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Review

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Breaking the epithelial polarity barrier in cancer: the strange case of LKB1/PAR-4

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The PAR clan of polarity regulating genes was initially discovered in a genetic screen searching for genes involved in asymmetric cell divisions in the *Caenorhabditis elegans* embryo. Today, investigations in worms, flies and mammals have established PAR proteins as conserved and fundamental regulators of animal cell polarization in a broad range of biological phenomena requiring cellular asymmetries. The human homologue of invertebrate PAR-4, a serine–threonine kinase LKB1/STK11, has caught attention as a gene behind Peutz–Jeghers polyposis syndrome and as a bona fide tumour suppressor gene commonly mutated in sporadic cancer. LKB1 functions as a master regulator of AMP-activated protein kinase (AMPK) and 12 other kinases referred to as the AMPK-related kinases, including four human homologues of PAR-1. The role of LKB1 as part of the energy sensing LKB1-AMPK module has been intensively studied, whereas the polarity function of LKB1, in the context of homoeostasis or cancer, has gained less attention. Here, we focus on the PAR-4 identity of LKB1, discussing the weight of evidence indicating a role for LKB1 in regulation of cell polarity and epithelial integrity across species and highlight recent investigations providing new insight into the old question: does the PAR-4 identity of LKB1 matter in cancer?

1. Introduction

The famous narrative from the Scottish author Robert Louis Stevenson, *The Strange Case of Dr Jekyll and Mr Hyde*, keeps inspiring not only those fascinated by psychiatric split personality disorder but also cell biologists working on multi-functional proteins. In this sense, the tumour suppressor protein LKB1/PAR-4 is a strange case. The LKB1 identity of this kinase protein, also known as serine/threonine kinase 11 (STK11), has been associated with upstream activation of the two isoforms of AMP-activated protein kinase (AMPK) and 12 AMPK-related kinases (ARKs). Doubtless, LKB1 is a key regulator of cell metabolism. However, LKB1/STK11 has yet another identity because the protein is a human homologue of PAR-4, a member of a PAR clan of proteins involved in regulation of cell polarity in worms, flies and mammals. Which, among the multiple identities of a single protein LKB1/STK11/PAR-4, is then Dr Henry Jekyll and which is the evil Mr Edward Hyde? The answer is obvious: none is Mr Hyde because cells do not express evil proteins. However, it appears that the bad nature of Mr Hyde surfaces on the pathways that suffer from loss of LKB1. Inheriting a mutated copy of the LKB1 gene predisposes to autosomal-dominant Peutz–Jeghers polyposis syndrome (PJS) and multiple cancers. LKB1 is also mutated in a variety of sporadic cancers. When cells lose LKB1, they also lose a mechanism to activate AMPK. Without AMPK activity, cells are not capable of controlling the mammalian target-of-rapamycin (mTOR) pathway, and consequently cells lose their grip on growth control and proliferation. This chapter about LKB1 is almost like something from the Stevenson story—Dr Jekyll is not inherently a good person but he is not acting like Mr Hyde because he is able to repress impulses, which feed the savage behaviour of Mr Hyde. How does the loss of PAR-4 identity become visible in tumour-prone cells that have lost LKB1? Investigations in flies and mammals have shown that cells without PAR-4 (LKB1) activity suffer from an inability to maintain apico-basal polarity and to sustain integrity of cell–cell

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junctions, including apical junctions. Damage at apical tight junctions in these cells may be a deed of Mr Hyde, because loss of cell cohesion promotes invasion of the cancer cells into neighbouring tissues. However, we posit in this review that one witnesses the true savageness of Mr Hyde in a ferocious assault that PAR-4-derepressed pathways launch against the basal side of the cells. The attack shatters the basement membrane using a transmembrane serine protease called Hepsin—akin to the heavy cane that Mr Hyde uses to beat his first victim to death. Basement membrane is the last line that cancer cells have to cross before they can invade connective tissue.

We first introduce here a few basic concepts of cell polarity and epithelial integrity, discussing how impaired control of these mechanisms may promote tumorigenesis. Thereafter, we focus on LKB1, discussing the relevance of the PAR-4 identity of LKB1 in polarity regulation and cancer.

2. Loss of epithelial polarity in cancer: an epiphenomenon or a cause?

Epithelial tissues vary in form and function, but the basic principle of apico-basal polarity is similar in different tissues. In polarized epithelial cells, the apical surface is oriented towards the lumen or external environment. This side of the epithelial cell, which often has membrane protrusions (microvilli), takes care of the absorption, exchange and secretion of molecules and macromolecules [1]. Lateral surfaces of the epithelial cells contact adjacent cells via specialized cell–cell junctions, namely tight junctions, adhesion junctions and desmosomes. On the opposite side of the apical membrane is the basal surface, which anchors cells to a basement membrane. Basement membrane is a thin (about 100 nm), dense sheet composed of a meshwork of insoluble molecules including laminin polymers, a cross-linked network of collagen IV fibrils, proteoglycans and glycoproteins [2,3].

(a) Epithelial cell polarity: frame and function

Polarized membranes and cytosolic molecular asymmetries are fundamentally important for epithelial cells to function as a multi-cellular organized tissue. The molecular asymmetries guide directional exocytosis and secretion of digestive enzymes from pancreas, milk from mammary epithelium and vectorial transfer of nutrients across the gut epithelium to blood [1]. Furthermore, extension of epithelial monolayers requires that cell divisions occur in the plane of the sheet and many key cell polarity proteins are involved in determining alignment of the mitotic spindle so that it is perpendicular to the axis of apico-basal polarity [4–6]. In addition to many proteins, lipids are also asymmetrically distributed in polarized cells [1]. In particular, the asymmetric distribution of phosphatidylinositol phosphates Ptd(4,5)P2 (PIP2) and Ptd(3,4,5)P3 (PIP3) along the apico-basal axis, observed in several cell types, has been attributed to the differential activities of PTEN phosphatases and PtdIns3-kinases (PI3K) on the apical and basolateral sides of cells [1,7,8]. Developing (primordial) adhesion junctions, tight junctions, desmosomes and basal cell surface–basement membrane contacts provide landmarks and orientation cues for development of apico-basal cell polarity [1,9]. Tight junctions maintain the polarized status of membrane domains by physically restricting lateral diffusion of integral membrane proteins. Also, key cadherins of adhesion junctions and desmosomes, E-cadherin and desmocollin, are necessary for proper formation of cell polarity and organization of epithelial structures [10]. Furthermore, there is evidence indicating that hemidesmosomes, which are structures attaching the basal surface of the cells to the underlying basement membrane at irregular intervals, are important for development of polarized epithelial architecture [11]. At the basal side of the cells, interference of β1-integrin contact with laminin can even invert the apico-basal polarity so that the apical surface becomes oriented towards the matrix [12]. The vesicular trafficking pathways, which are often guided by distinct cytoskeletal tracks, are important cellular machineries for maintenance of polarity as they direct and recycle plasma membrane proteins specifically to the apical membrane and basement membrane [9,13,14]. For example, synthesis of functionally active basement membrane requires both polarized localization of plasma membrane proteins, for example integrins, and polarized secretion of basement membrane proteins, for example laminins [3,15].

In *Drosophila* and mammals, the apical identity of apico-basally polarized cells is maintained and regulated by two conserved polarity complexes (named according to gene names), the CRB/PALS1/PATJ (Crb) complex and the PAR3/PAR6/aPKC (Par) complex. On basolateral sides of the cells, a module of Scrib, Dlg and Lgl proteins controls the basolateral identity [16]. Scrib, Dlg and Lgl physically interact with each other in *Drosophila* epithelial cells, forming a Scrib complex, whereas in mammalian cells, the nature of these interactions is less clear. The core molecular machinery that generates cellular asymmetry is conserved from worms to mammals. The main components of the machinery are six (or five depending on species) functionally, but not structurally, related PAR (for 'partitioning defective') proteins [17]. The core set of PAR proteins, which is discussed in §3, along with a limited number of other proteins such as aPKC and CDC42, is involved in a broad range of phenomena requiring cellular polarization, such as apico-basal polarity, neurite extension, cellular migration and asymmetric cell division.

(b) Epithelial cell polarity: collapse in cancer

Cancer progression from benign tumour (local mass of cells) to invasive and metastatic cancer features loss of all aforementioned characteristics of polarized epithelial cells. Indeed, loss of organized epithelial structure, loss of cell polarity and loss of basement membrane attachment are among the key diagnostic criteria that differentiate benign tumours from life-threatening malignant cancers. One could envision that collapse of the polarity system benefits the process of cancer progression in many ways (figure 1). For example, erratic alignment of the mitotic spindle could enable efficient expansion of a cell mass in every direction, thus promoting hyperplasia. Out-of-alignment mitotic spindle may also increase aneuploidy [5,23]. Altered cell adhesion and extracellular matrix-dependent signalling mechanisms may make cells more migratory [24], loss of lipid asymmetry may deregulate spatial PI3K signalling and any cell-intrinsic (e.g. loss of cues for directional secretion) or -extrinsic mechanism harming basal polarity could lead to deterioration of basement membrane, thus paving the way to invasion and metastasis [24,25]. Thus, in general, the aforementioned qualities would

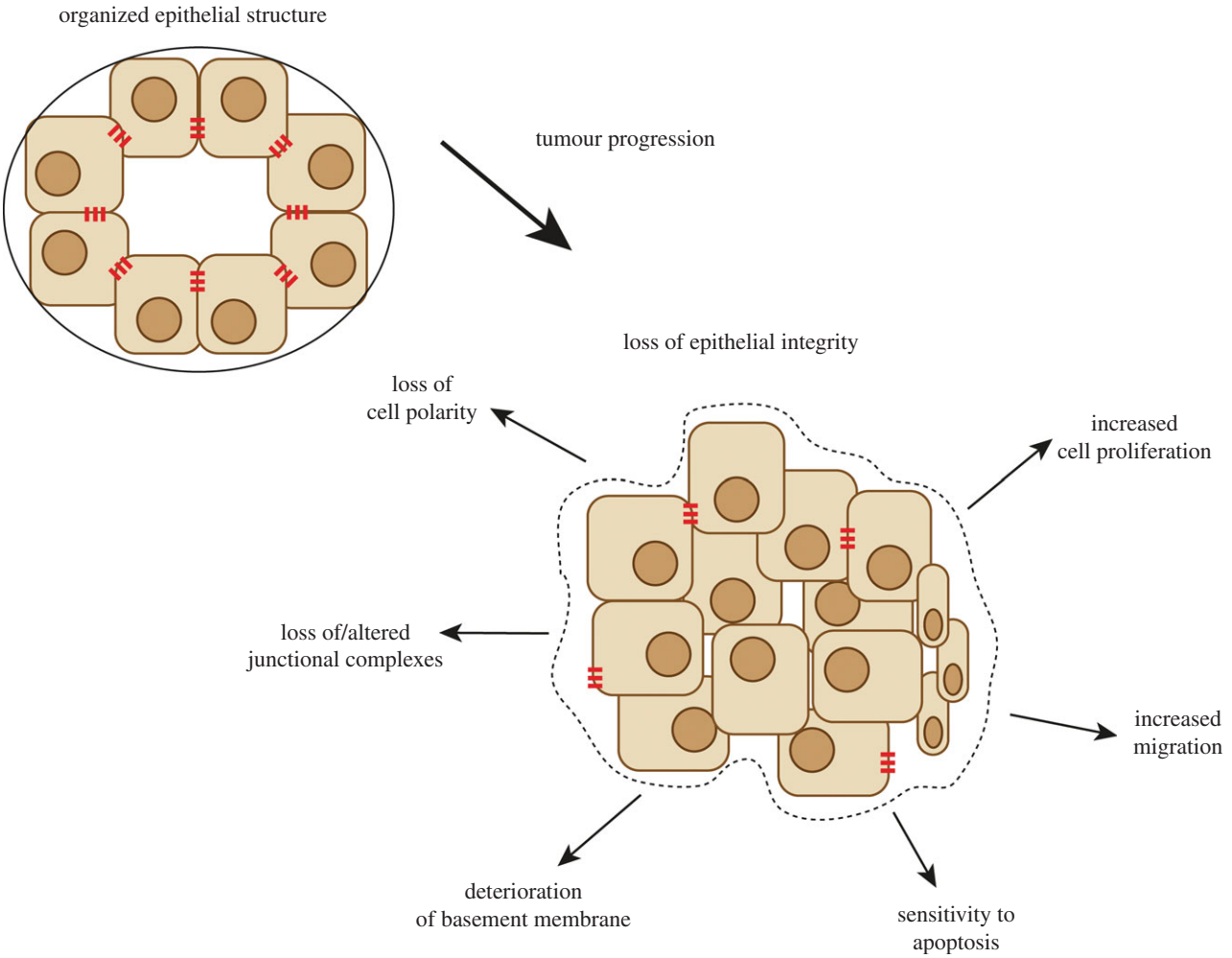

Figure 1. Loss of epithelial integrity—a hallmark of all advanced cancers. A schematic of cellular level changes, which typify disintegration of epithelial structure during tumour progression. Partial or complete loss of basement membrane (BM) is a defining diagnostic marker to distinguish the *in situ* type of carcinoma from invasive carcinoma [18]. Loss of cell–cell junctions, for example via loss of E-cadherin, is a common feature associating with the epithelial–mesenchymal transition [19,20]. Junctional complexes are also altered so that adhesive cell–cell junctions are loosened and migratory focal adhesions increased [19]. Loss of epithelial cell polarity associates with deterioration of cell junctions and basement membrane, as discussed in this review, which enhances the proliferative potential and migratory capacity of epithelial cells [21]. However, the cell polarity machinery cannot be completely demolished in cancer, because the core polarity machinery is necessary not only for apico-basal polarity but also for cell migration [22]. Thus, evolving cancers may need to carefully select polarity targets so as to acquire benefits from loss of apico-basal polarity but not to compromise ability to proliferate or migrate.

benefit arising tumours by cutting off cell structures, which stabilize organized epithelial structure (cell–cell adhesions, basement membrane), and endowing cells with new migratory capacities to move out of epithelial organizations and into the stroma.

The polarized phenotype of epithelial cells is lost when a tumour progresses towards malignancy, but it is still unclear whether there are specific and prevalent genetic mutations that contribute to tumour progression because they disrupt epithelial cell polarity. So far, experiments in *Drosophila* provide the strongest evidence for a causal role of polarity genes in tumour progression. The genes of the Scrib complex form the core of *Drosophila* neoplastic tumour suppressor genes (nTSGs) and, beyond nTSGs, inactivation of almost any core gene of the polarity machinery, for example *bazooka* (equivalent of human PAR3), *stardust* (similar to human PALS1) and *cdc*42, dramatically promotes metastatic behaviour of Ras or Raf-initiated tumours [16,26,27].

However, these findings in *Drosophila* may not directly translate into human cancers, because many recently published reports cataloguing the most frequently mutated genes across thousands of human cancer genomes do not feature polarity genes at the top of the lists. Our own investigation to estimate the frequency of somatic mutations in polarity genes, several years ago, suggested that these mutations are indeed rare [21]. The rarity of mutations in core polarity genes, however, does not mean that cell polarity would be an irrelevant concept in cancer. In mammals, genetic redundancy efficiently buffers against deleterious effects of single-gene mutations, and there are commonly multiple homologues corresponding to prototypic *Drosophila* polarity genes [28]. This means that, in mammals, tumour microevolution could preferentially single out non-redundant polarity genes (rather than the most typical) as targets for polarity-damaging mutations. It is also worth noting that recent focused screens on cancer cell lines for small intragenic deletions have identified hitherto missed mutations in polarity genes, for example in PAR3, paving the way for more precise analysis of polarity-gene mutations in heterogenous primary tumour material [29]. Further evidence supporting the role of polarity genes as potential tumour suppressor genes comes from the findings that several core polarity genes, for example Dlg and Scrib,

are targeted for degradation by oncogenic human papillomaviruses [30]. Finally, in mouse models of human cancer, like in *Drosophila* models of tumour progression, a cell polarity gene deficiency (LKB1/PAR4, SCRIB, PAR3) can dramatically promote tumorigenesis in tissues engineered to express a dominantly acting oncogene, such as Myc or Ras [31–34].

The role of core polarity genes and surrounding regulatory networks in cancer has been recently comprehensively covered in many reviews. This emerging field of research continues to inspire investigators as it holds promise for identifying novel, fundamentally important pathways contributing to tumour progression and invasion as well as new therapies to fight cancer [7,9,34–36]. This review focuses on liver kinase B1, serine–threonine kinase 11 (LKB1/STK11), which is a human homologue of PAR-4. LKB1 has the strongest link to cancer among the PAR proteins but it is also a multi-functional protein with a number of other functions, besides polarity regulation.

3. The LKB1 tumour suppressor gene: PAR-4 and more

The LKB1 gene is the only human homologue of *Caenorhabditis elegans* par-4 and *Drosophila lkb1*, encoding a serine–threonine kinase with multiple functions in regulation of cell polarity, metabolism and cell growth [37–39]. LKB1 is known as the critical upstream kinase required for the activation of AMPK, which is a cellular energy sensor and central regulator of cell metabolism. The LKB1-AMPK module also operates via the mTOR pathway to regulate cell growth [38]. These aspects of LKB1 signalling and links of the pathways to cancer have been covered in a number of recent reviews [37–39]. Below, we review the incidence of LKB1 mutations in cancer and discuss the PAR-4 identity of LKB1, illuminating functions and signalling of LKB1 in the context of physiological regulation of cell polarity and epithelial tissue integrity.

(a) LKB1 in hereditary and sporadic tumours

Germline mutations in tumour suppressor LKB1 lead to autosomal-dominant PJS, characterized by benign hamartomatous polyps occurring throughout the gastrointestinal tract [40–42]. Patients with PJS also have a significantly increased risk of developing cancer, for example, in the gastrointestinal tract, pancreas, breast and ovaries [43–45].

Different types of sporadic cancers also harbour somatic mutations in the LKB1 genes. Most frequently, LKB1 mutations have been found in non-small cell lung carcinoma. In this cancer type, more than 30% of the cases have somatic and homozygous inactivating mutations in LKB1 [46,47]. Somatic LKB1 mutations are also common in cervical cancer, found in about 20% of cases [48]. In addition, LKB1 mutations have been observed in melanomas and cancers of the pancreas, liver, breast and endometrium, although at reduced frequency [49–53]. Reduced or missing expression of Lkb1 has been reported in a range of cancers, including endometrial, pancreatic and breast cancer [49,54–56]. In breast cancer, LKB1 mutations appear to be rare, but loss of expression is common based on the results from tissue microarrays and Western blot analysis of tumours [32,54]. Loss of LKB1 expression has been attributed to epigenetic silencing mechanisms, including promoter hypermethylation in breast cancer, and there is also some evidence that reduced LKB1 levels correlate with poor prognosis in these cancers [54,57,58]. In melanoma, posttranslational inactivation mechanisms may target LKB1 because mutated B-RAF leads to phosphorylation of LKB1, which inhibits it from activating downstream targets [59].

(b) LKB1 as a polarity protein PAR-4

The PAR (partitioning defective) set of polarity regulating proteins was initially found through a screen searching for mutants that disrupt the first asymmetric cell divisions in the *C. elegans* embryo [60]. PAR-4, the *C. elegans* homologue of LKB1, was among the six PAR genes found necessary for cytoplasmic partitioning and asymmetric cell division in the worm zygote (figure 2). Subsequent studies have exposed PAR proteins as fundamental regulators of cell polarization in diverse animals and different contexts of polarity [17]. The core PAR proteins are a diverse group of proteins: PAR-1 and PAR-4 encode serine–threonine kinases, PAR-5 belongs to the 14-3-3 family of proteins, which are recruited to phosphorylate serines and threonines. PAR-3 and PAR-6 contain PDZ domains, which are signalling and protein interaction scaffolds, and PAR-2 has a RING finger domain that may be used in the ubiquitination pathway. PAR proteins 1, 2, 3 and 6 acquire asymmetric localization patterns during the development of cell polarization in *C. elegans*, whereas PAR-4 remains symmetrically localized in cortex and cytoplasm during the process [17]. Interestingly from the LKB1 perspective, epistatic analyses have demonstrated that PAR-4 is essential for establishment of PAR-3/PAR-6 asymmetry, suggesting that PAR-4 may master-regulate the asymmetric segregation of other PAR family proteins [40,61].

In *Drosophila* (figure 2), PAR-4/*lkb1* is required for the formation of early anterio-posterior polarity in oocytes and epithelial apico-basal polarity in follicle cells [62]. In adult *Drosophila* retinal cells, inactivation of *lkb1* also leads to disruption of polarity and further disarrangement of cell–cell junctions and membrane domains [63]. The signalling circuitries, which in flies may operate downstream of *lkb1* in epithelial polarity regulation, are discussed below.

In mammalian cells (figure 2), construction of a STRAD adaptor protein-mediated system to ectopically activate LKB1 by Baas *et al.* [64] led to a surprising finding that activation of LKB1 is sufficient to polarize single, isolated intestinal epithelial cells in culture—a finding that challenged current views of the importance of cell–cell contacts in the process of polarization. Subsequent descriptive and functional studies have pictured LKB1 as a cell-polarity-linked protein in various contexts of mammalian tissue asymmetries. For example, LKB1 has been implicated in polarization of mouse oocytes [65]. Furthermore, LKB1 is required for axon specification during polarization of cortical neurons [66,67] and it participates in the formation of Sertoli cell polarity and testicular junctions [68].

Recent studies from our laboratory have also suggested that LKB1 is crucial for the integrity of mammary epithelial tissue [32,69]. A detailed analysis of three-dimensional cultures of primary mouse mammary epithelial cells (MMECs), rendered LKB1 deficient by adenoviral Cre infection, shows that loss of LKB1 disorganizes the normally rounded phenotype of acinus-like structures. Characteristic of LKB1-deficient structures is lateralized or completely mislocalized expression of apical polarity markers. In an electron microscopic analysis,

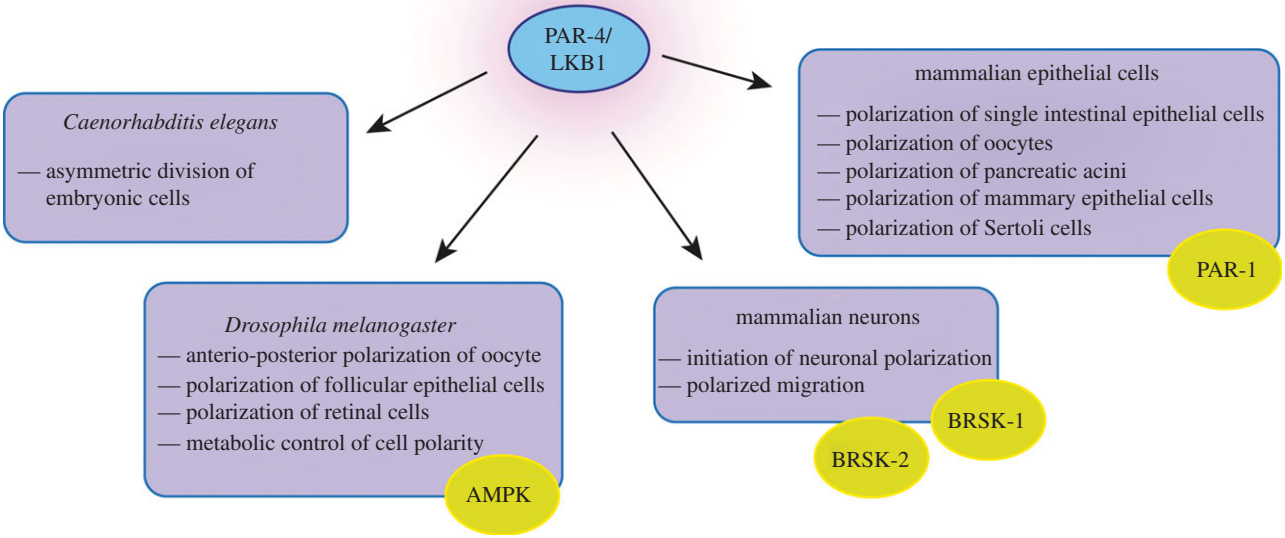

Figure 2. LKB1/PAR-4 in regulation of cell polarity. LKB1 functions as a PAR-4 cell polarity gene in *C. elegans*, *Drosophila* and mammalian epithelial and neuronal cells. Highlighted are suggested downstream targets, through which LKB1 may regulate cell polarity in different systems.

the LKB1-deficient three-dimensional structures show multiple abnormalities in desmosomes and tight junctions as well as deteriorated basement membrane. A selective deletion of LKB1 in the adult mammary gland, obtained by crossing mice with floxed LKB1 alleles with mice harbouring a lactogenic hormone-inducible WAP-Cre construct leads to many similar phenotypes observed in the *in vitro* three-dimensional cultures. The LKB1-deficient mammary ductal and alveolar structures express a disorganized pattern of apical markers and incomplete basement membrane as well as displaying enhanced branching of the ducts [32]. However, inactivation of LKB1 may lead to strikingly different phenotypes in different epithelial tissues as discussed next.

(c) LKB1 and epithelial tissue morphogenesis

The basic architecture of epithelial tubes in all branching organs, for example, kidney, mammary gland, lung and blood vessels, is a polarized epithelium that apically faces a central luminal space and basally contacts basement membrane [70]. The mechanisms of branching are complex and vary between different tissue types, but the main underpinnings of the branching process are coordinated actions between proliferation, apico-basal polarity and epithelial motility machineries. Several investigations have also highlighted the role of basement membrane as a scaffold providing cues for reorientation of polarity during the branching process as well as incomplete basement membrane coverage at branching points as an element regulating branching morphogenesis [70].

Recent investigations have coupled LKB1 to branching morphogenesis in two different organs—lung and the mammary gland. The study of Lo *et al.* [71], using an ATP-binding pocket modified form of LKB1 that confers sensitivity to specific inhibition by a small molecule ATP analogue, shows that inactivation of the modified LKB1 inhibits branching morphogenesis of embryonic lung tissue in an *ex vivo* explant culture. The branching defect was rescued by activation of AMPK, suggesting a role for the LKB1-AMPK pathway in the branching process. The same study also shows that the phenotypic effects resulting from inactivation of LKB1 are tissue- and context-specific. In pancreatic tissue, chemical inactivation of LKB1 results in development of cystic structures by mechanisms not involving defective AMPK signalling.

In the study of Partanen *et al.*, conditional deletion of LKB1 genes, *ex vivo*, in three-dimensional organoids and, *in vivo*, in the mammary gland, leads to hyperbranching of the mammary ducts. In the mammary gland, loss of LKB1 simultaneously leads to defects in cell polarity, cell junctions and basement membranes [32]. In the study of Lo *et al.* [71], polarity defects were not observed in embryonic lung tissue after inactivation of LKB1. Therefore, it is possible that the hyperbranching phenotype results from loss of polarity and incomplete basement membrane coverage, and in tissues spared from these defects, LKB1 loss may have other, even opposite effects.

(d) LKB1/PAR-4 signalling pathways in epithelial polarity

The kinase activity of LKB1 in mammalian cells requires the formation of a heterotrimeric complex consisting of LKB1, pseudokinase Ste20-related adaptor protein (STRAD) and a scaffold protein, for example mouse protein 25 (MO25) [72,73]. Binding to STRAD and MO25 renders LKB1 active and furthermore, regulates stability and localization of LKB1 protein [73,74]. LKB1 functions as a master regulator of the two isoforms of AMPK (AMPK-α1 and AMPK-α2) and 12 other kinases referred to as the ARKs [75–78]. All LKB1-regulated downstream kinases, AMPK and ARKs, require LKB1-dependent phosphorylation in the threonine 172 (T172) (or equivalent residue) that resides in the activation loop to become active. The AMPK and ARK serine–threonine kinases belong to a small subfamily of calcium/calmodulin-dependent protein kinases comprising AMPK-α1 and AMPK-α2, four MARK/PAR-1 kinases (MARK1-4), the AMPK-related kinase 5 (ARK5, NUAK1), the SNF/AMPK-related kinase (SNARK, NUAK2), two Brain-specific kinases (BRSK1 and 2), three

salt-induced kinases or SIKs (SIK1/SIK, SIK2/QIK and SIK3/QSK) and the Snf-related serine/threonine kinase (SNRK) [76].

Among the LKB1 downstream kinases, AMPKs, MARK/PAR-1 kinases and BRSK1 and 2 have been linked to regulation of cell polarity in a context-dependent manner (figure 2). Findings in *Drosophila* have suggested that LKB1 may use AMPK to signal cell polarity [79]. In *Drosophila* follicular epithelial cells, *ampk*-α null mutants exhibit normal epithelial polarity in a nutrient-rich environment but in culture media deprived of sugars, conditions where AMPK normally is maximally active, apico-basal cell polarity of these cells is disrupted. Also *lkb1* mutants show similar energy-starvation-dependent polarity defects, which can be rescued by a constitutively active AMPK transgene [79]. Another study has shown that downstream of the *ampk*-α mutation, a constitutively active form of a protein non-muscle myosin regulatory light chain (MRLC or MLC2) rescues the polarity defects of *ampk*-α mutant cells [80]. These investigations altogether suggest that at least in flies, the LKB1-AMPK-MRLC module regulates cell polarity in a mode that is responsive to cues from cellular energy status. In mammals, branching defects in embryonic lung tissue, caused by LKB1 inactivation, can be rescued via ectopic activation of AMPK [71]. In cultured mammalian cells, AMPK has also been shown to play a role in cell polarity and formation of tight junctions [81,82].

Mammalian microtubule-associated protein (MAP)/microtubule affinity-regulating kinases (MARKs) are homologues of *C. elegans* PAR-1, which was identified in the same *C. elegans* partitioning defective screen as LKB1/PAR-4 [60]. PAR-1 serine/threonine kinase regulates other PAR proteins to establish cell polarity. For instance, in *Drosophila*, PAR-1 phosphorylates PAR-3 to retain it in the apical pole of the oocyte [83]. In mammals, MARK2 (PAR1b) has been implicated as a downstream effector of LKB1 in regulation of apico-basal polarity of pancreatic acinar cells *in vivo* [84]. Recently, it was found that MARK2 physically associates with a RhoA-specific guanine nucleotide exchange factor, influencing RhoA activity and the actin cytoskeleton. This interaction could couple PAR-1-dependent polarity regulation to cell movement [85]. The virulence factor CagA in *Helicobacter pylori* cagA-positive strains interacts with MARKs/PAR1 causing junctional and polarity defects. It is believed that this interaction leads to the disorganization of epithelial architecture and loss of epithelial barrier function observed in *Helicobacter*-infected gastric epithelium [86]. MARKs may also regulate cell polarity by phosphorylating MAPs, for example tau, and that type of interaction is important for polarized protein trafficking [87,88].

In neuronal cells, polarization is important for axonal and dendritic specification that is fundamental to the ability of neuronal cells to transmit information. LKB1 has been linked to regulation of polarized migration of neurons and to promotion of axon initiation during neuronal polarization through downstream kinases BRSK1 and -2 (also known as SAD-A and -B), which in turn may contribute to cell polarity via phosphorylation of MAPs [66,67,89].

4. Loss of PAR-4 identity matters in cancer: evil deeds on the basal side of the epithelial cells

As discussed in the beginning of this review, collapse of a cell polarity system can benefit cancer progression in multiple ways. However, among the pillars of epithelial integrity, the basement membrane is the last barrier to resist invasion of motile cancer cells into the neighbouring tissues. Therefore, any cell-intrinsic or -extrinsic mechanism harming the integrity or altering the biological activity of basement membrane could promote invasion and progression to metastatic disease [24,25,90]. Loss of LKB1 has been shown to influence the biological activity of basement membrane via non-proteolytic and proteolysis-dependent mechanisms. The functional interaction between LKB1 loss and type II transmembrane proteases may also involve polarity regulation.

(a) LKB1 and non-proteolytic moulding of basement membrane

A stiffening of the extracellular matrix, which is attributable to increased or inappropriate cross-linking of collagens, contributes to increased migratory and invasive behaviour of cancer cells and is considered one indicator of poor cancer prognosis [91–93]. One contributing factor to matrix stiffening is enhanced activity of lysyl-oxidases (LOX), enzymes that play an important physiological role in strengthening collagen fibrils, including type IV collagen of basement membrane, via covalent cross-linking of lysine residues in these proteins [93]. A functional role of LOX in cancer has been addressed by blocking LOX activity, which reduces tumour incidence in the MMTV-Neu mouse model of breast cancer [93]. Loss-of-function mutations in LKB1 are common in lung cancer and loss of LKB1 has been observed to correlate with significantly enhanced levels of LOX enzymes. In a mouse model of lung cancer and human non-small cell lung cancer cell lines, loss of LKB1 leads via enhanced mTOR and HIF1a signalling to enhanced LOX activity, increased deposition of collagen and enhanced proliferation possibly caused by increased matrix stiffness [94]. Thus, although the physiological significance of LKB1–LOX crosstalk remains unclear, loss of LKB1 and the consequent dysregulation of LOX activity in the context of tumour tissue has a prominent impact on stromal extracellular matrix remodelling and collagen biology (figure 3).

(b) LKB1 and proteolytic moulding of basement membrane

(i) LKB1 and metalloproteinases

Matrix metalloproteinases (MMPs) are a group of enzymes able to degrade various extracellular matrix and cell surface molecules, aiding in tissue remodelling and branching morphogenesis. The activity of MMPs is commonly elevated in cancers [95–97]. However, whether these enzymes specifically contribute to degradation of collagen IV, a basement membrane transmigration-limiting factor [98], is unclear although MMP-2 and MMP-9 have been implicated in the process [95,99]. In a study, LKB1 overexpression has been shown to correlate with downregulation of MMP-2 and MMP-9 and invasion inhibition in an *in vitro* assay as well as with the tumorigenicity and metastasizing capacity of xenografted tumour cells [100]. However, the significance of these findings in the context of LKB1 inactivation remains to be determined.

(ii) LKB1 and type II transmembrane serine proteases

In our study, using mice with LKB1-deficient mammary glands, we found evidence that basement membrane may

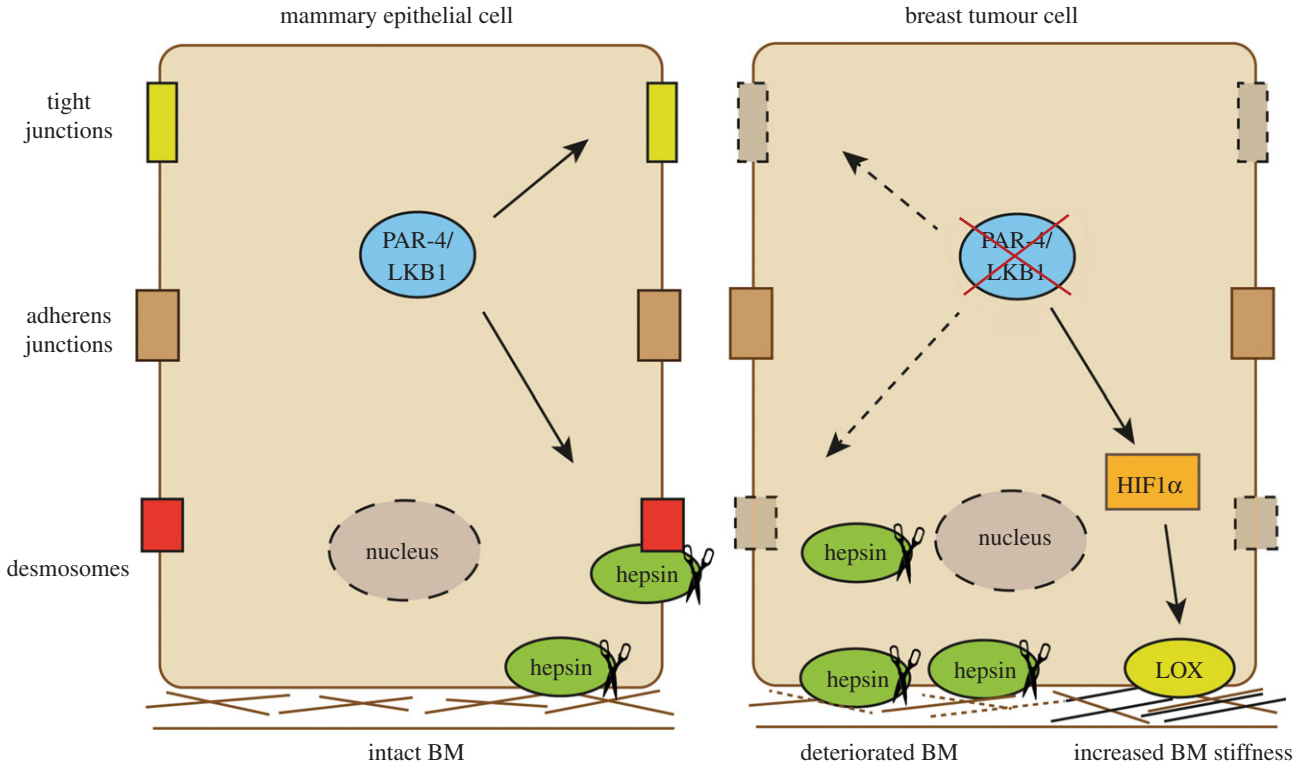

Figure 3. A model: loss of LKB1 and basement membrane remodelling. Mammary epithelial cells are bound to each other via tight junctions, adhesion junctions and desmosomes. A TTSP hepsin is expressed on the basal and lateral membranes of epithelial cells, exhibiting a colocalized expression pattern with desmosomes. Loss of LKB1 inflicts damage to tight junctions and desmosomes, which associate with widespread loss of epithelial integrity. In the present model (modified from Partanen [32]), this damage liberates hepsin from the membranes into the cytosol. Hepsin is commonly overexpressed in cancer (an effect that can be seen in cells engineered to overexpress MYC), and we posit that the overexpressed and mislocalized hepsin causes deterioration of basement membrane (BM). Breakdown of BM is considered an essential step for tumour invasion and metastases. However, loss of LKB1 may also exert opposite, stabilizing effects on BMs and extracellular matrix by stimulating expression of the collagen cross-linking enzyme LOX [94]. Also, these effects may benefit tumour progression, at least once the tumour has breached the BM barrier, by increasing the stiffness of the extracellular matrix. Stiff matrix stimulates tumour cell proliferation and, in some instances, migration. The investigations expose the complex nature of functional interactions between LKB1 and constituents of extracellular matrix, which may inhibit or facilitate tumorigenesis depending on context.

be degraded by type II transmembrane serine proteases (TTSPs) during mammary tumorigenesis [32]. We reported that loss of LKB1 does not induce palpable tumours during the lifetime of the mice but leads to mild abnormalities in the mammary gland, such as hyperbranching, partial disorganization of apical polarity and incomplete basement membrane. However, combining LKB1 deficiency with oncogenic MYC dramatically accelerates the appearance of mammary tumours in comparison with glands exposed to oncogenic MYC alone [32]. The study shows that both MYC^+ and $MYC^+;LKB1^-$ tumours are histopathologically mostly adenocarcinomas and not informative as such regarding the possible mechanisms underlying accelerated tumorigenesis. However, the histology of hyperplastic regions in between tumour areas was particularly interesting, providing a path for follow-up studies. Expression of oncogenic MYC alone, a stimulus known to promote cell proliferation, had led to development of surplus ductal branches and alveolar units. All structures generated under the influence of MYC were separated from stroma and neighbouring epithelial structures by basement membranes. By contrast, the hyperplastic regions in the $MYC^+;LKB1^-$ mammary glands had surplus epithelial cells but mostly unrecognizable ductal or alveolar epithelial structures. In the hyperplastic regions of $MYC^+;LKB1^-$ mammary glands, the bordering basement membranes were missing giving the impression that epithelial structures were fused together into a huge disorganized cell mass. Missing basement membrane was a defining difference between the hyperplastic glands expressing oncogenic MYC (basement membrane present) and those expressing MYC combined with loss of LKB1 (basement membrane absent).

Investigating MMECs, isolated from aforementioned mice, in *ex vivo* three-dimensional cultures, we observed that MYC^+ epithelial structures were hyperplastic but still maintained the rounded morphology. By contrast, the $MYC^+;LKB1^-$ structures showed extensive branching and lacked basement membrane components, such as nidogen and collagen IV [32]. A panel of small molecule inhibitors tested in a rescue assay did not support specific roles for MMPs, but instead suggested involvement of TTSPs in deterioration of basement membrane. Subsequent studies identified a strong correlation between loss of LKB1 and overexpression of a TTSP called hepsin/TMPRSS1 in our transgenic mouse models and in a panel of 60 clinical human breast cancer samples. Knockdown of hepsin rescued basement membrane in $MYC^+;LKB1^-$ three-dimensional structures, indicating a role for hepsin in basement membrane degradation [32].

While the question of whether, downstream from LKB1 loss, hepsin-mediated deterioration of basement membrane promotes mammary tumorigenesis has not been addressed, the oncogenicity of hepsin has been investigated specifically in prostate cancer models. Hepsin originally caught the

attention of cancer researchers in microarray profiling studies, who found almost ubiquitous overexpression of hepsin in prostate cancer [101,102]. Nearly 90% of prostate cancers overexpress mRNA for hepsin with up to 30-fold levels compared with normal prostate tissue. Profound hepsin overexpression is also found in ovarian and breast cancers, and high hepsin mRNA levels are commonly accompanied by strong hepsin protein signal in tumour sections [32,103–106]. A mouse model engineered to express hepsin under probasin promoter in prostate has revealed widespread defects in the integrity of hemidesmosomes and basement membrane, indicating deleterious interaction between hepsin overexpression and basement membrane *in vivo* [107]. *In vitro*, the targets for the proteolytic activity of hepsin include the major basal laminal component laminin-332 [108]. Basement membrane degradation is a critical step for initiation of cancer metastasis, and evidence from transgenic and orthotopic tumourgraft models of prostate and ovarian cancer suggest that overexpression of hepsin can promote incidence of metastases [104,107,109]. However, the relationship between LKB1-deficiency and hepsin overexpression in prostate cancer has not yet been systematically studied. According to the data obtained from the Wellcome Trust Sanger Institute Cancer Genome Project web site, http://www.sanger.ac.uk/genetics/CGP (COSMIC and CONAN), LKB1 mutations have been found in prostate cancer cell lines and with less than 1% frequency in prostate cancer but to our knowledge, the epigenetic influences on gene expression levels have not yet been addressed in prostate cancer.

(c) Hepsin: permanent desmosome resident or a cleaver-wielding bandit on the loose

It is still unclear how mechanistically overexpression of hepsin damages basement membrane, but investigations on the localization of hepsin in normal and transformed cells, and changes in this localization in response to oncogenic signalling may provide some clues. In non-transformed and some transformed cells, the transmembrane protease hepsin localizes to cell membranes, its protease domain pointing to the pericellular space [110]. In ovarian cancer cells and primary MMEC, the immunostaining pattern of hepsin prominently overlaps with desmosomal proteins [32,104]. Desmosomes are junctional complexes mediating the attachment of specific cell-surface adhesion proteins, desmogleins and desmocollins, to intracellular intermediate filaments. In general, tumours express either a membranous or cytosolic pattern of hepsin depending on the type and grade of the tumours [32,101,104,105].

Absence of critical desmosomal proteins, for instance desmoplakin, or depletion of desmoplakin in response to LKB1 inactivation leads to translocation of hepsin from cell–cell borders to cytosol [32,104]. In ovarian cancer cells, also, the ectopically overexpressed hepsin is mainly cytosolic [104]. Therefore, it can be hypothesized that hepsin is normally sequestered to desmosomes but if the junctional integrity is compromised, as in most cancer cells, hepsin becomes liberated in the cytosol (figure 3). It still remains a mystery how cytosolic hepsin interacts with basement membrane but it is interesting to note that vesicular trafficking plays an important role in the activation cascades of other TTSPs. A TTSP family member, matriptase has been reported to activate a downstream TTSP prostasin on the basolateral membrane of polarized colonic epithelial cells from which activated prostasin is intracellularly transcytosed to apical membrane of the cells [111]. It is tempting to speculate that loss of cues for directional secretion in depolarized cancer cells perturbs cell polarity-coupled proteolytic cascades. Hence, disruption of polarity could lead to uncontrolled proteolytic attacks on different cellular domains, including basement membranes.

Finally, in addition to causing physical damage to basement membrane, overexpressed (or liberated) hepsin may also contribute to tumour progression via remodelling of basement membrane, which is not only a barrier but also a dynamic scaffold that controls access of the cells to various growth factors. For example, proteoglycans bind and sequester growth factors and cytokines, regulating their availability to cells [112,113]. Moreover, basement membrane proteins often contain cryptic sites, which are exposed after extracellular matrix digestion and can consequently act as pro-migratory, pro-invasive or angiogenic cues [2,114]. Hepsin can proteolytically activate many factors in the microenvironment of epithelial cells, including hepatocyte growth factor, urokinase-type plasminogen activator, macrophage-stimulating protein and EGF receptor, all of which are well-known players in cancer [110,115–120].

5. Conclusion: lessons learned and challenges ahead

Common interest in regulation and dysregulation of cell polarity in health and disease has recently brought together biologists with diverse backgrounds, representing single cell and invertebrate models, developmental and neurobiology, and cancer biology. In particular, past studies in *Drosophila*, revealing how potently loss-of-gene functions involved in cell polarity and epithelial integrity promote tumorigenesis, have spurred interest in the polarity pathways among cancer biologists. However, it is clear that the evolutionary distance and gene diversification between flies and humans makes it challenging to directly relate tumour genetics in flies to human cancer. For example, it is still a matter of debate whether the closest human structural homologues of fly nTSGs are prevalent in human tumour suppressor genes. While the ongoing studies on human versions of nTSGs are expected to shed light on the question, it is also emerging that the PAR clan of proteins plays an important role at the intersection of polarity regulation and tumour suppression in mammals. PAR-4/LKB1 is the clan member with a strongest link to cancer but the multiple functions of LKB1 makes it challenging to discern the role of the PAR-4 identity of LKB1 in tumorigenesis. PAR-4 also does not have asymmetric localization in cells, in contrast to for example apical PAR-3 and PAR-6, which makes it difficult to distinguish between apical and basal polarity-directed actions of PAR-4/LKB1. However, current studies have indicated evidence that loss of PAR-4/LKB1 weakens apical cell–cell cohesion and launches hepsin-mediated proteolytic and LOX involving non-proteolytic pathways to digest and remodel the basement membrane on the basal side of the cells as well as extracellular matrix—all of these events promote the spread of cancer cells from epithelial structures to connective tissue. While this evidence supports the involvement of the PAR-4 identity in tumour development, a strong case also exists to convict the LKB1-AMPK-mTOR branch of involvement in tumorigenesis. On which side does the burden of

proof lie? According to current knowledge, LKB1 predominantly signals via AMPK and the PAR-4 identity of LKB1 signals via activation of PAR1 (MARKs). However, how separate these identities in fact are is a question that will deserve closer inspection. PAR-1/MARK proteins belong to a family of ARKs and, in addition, evidence exists from studies in flies and mammals indicating the involvement of LKB1-AMPK axis in polarity regulation. The possibility that metabolic pathways interact with polarity pathways, or vice versa, warrants further studies as an area of obvious interest and significance to epithelial biology. This takes us back to Stevenson's novella, *The Strange Case of Dr Jekyll and Mr Hyde*. At the end of the story, the line between the different identities of Dr Jekyll and Mr Hyde becomes blurred when Dr Jekyll keeps metamorphosing into Hyde involuntarily and Hyde seeks a potion to revert back to Dr Jekyll again. If this chapter holds to the strange case of LKB1/PAR-4, future research will identify not only isolated phenotypes attributed to LKB1/PAR-4 but also signalling nodes linking different LKB1/PAR-4 regulated networks and cellular phenotypes. Such nodes would be prime targets for therapeutic potions aiming to change evil Hyde back to Jekyll again.

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