Mitotic spindle misorientation in cancer – out of alignment and into the fire

Jillian C. Pease and Jennifer S. Tirnauer*

Center for Molecular Medicine, University of Connecticut Health Center, Farmington, CT 06030-3101, USA

*Author for correspondence (tirnauer@uchc.edu)

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Summary

Mitotic spindle orientation can influence tissue organization and vice versa. Cells orient their spindles by rotating them parallel or perpendicular to the cell – and hence the tissue – axis. Spindle orientation in turn controls the placement of daughter cells within a tissue, influencing tissue morphology. Recent findings implicating tumor suppressor proteins in spindle orientation bring to the forefront a connection between spindle misorientation and cancer. In this Commentary, we focus on the role of three major human tumor suppressors – adenomatous polyposis coli (APC), E-cadherin and von Hippel-Lindau (VHL) – in spindle orientation. We discuss how, in addition to their better-known functions, these proteins affect microtubule stability and cell polarity, and how their loss of function causes spindles to become misoriented. We also consider how other cancer-associated features, such as oncogene mutations, centrosome amplification and the tumor microenvironment, might influence spindle orientation. Finally, we speculate on the role of spindle misorientation in cancer development and progression. We conclude that spindle misorientation alone is unlikely to be tumorigenic, but it has the potential to synergize with cancer-associated changes to facilitate genomic instability, tissue disorganization, metastasis and expansion of cancer stem cell compartments.

Key words: APC, E Cadherin, VHL, Spindle alignment, Spindle orientation

Introduction

Mitosis does more than segregate the chromosomes; it can also decide cell fate and tissue architecture. During mitosis, the spindle forms through the disassembly of microtubules and their reassembly into a highly ordered bipolar structure (for a review, see Mitchison and Salmon, 2001). Duplicated centrosomes become the spindle poles and a burst of microtubule polymerization creates the spindle. Some microtubules extend inward to form interpolar or kinetochore microtubules, and these two microtubule populations are responsible for aligning and segregating the chromosomes (Mitchison and Salmon, 2001).

A third population of microtubules – astral microtubules – extend outward from the spindle poles towards the cell cortex. These microtubules position and orient the entire spindle within the cell (Giansanti et al., 2001; O'Connell and Wang, 2000). Spindles can be positioned in the geometric center of the cell, as is seen in some cultured cells, or in an eccentric location, as is seen in early embryogenesis and in the well-studied *Drosophila* neuroblast system (Gönczy et al., 1999; Green et al., 2005; Kim et al., 1997; O'Connell and Wang, 2000; Roegiers et al., 2001; Wright and Hunter, 2003). Spindle displacement away from the cell center can be used to generate daughter cells of unequal sizes (Kaltschmidt et al., 2000).

Astral microtubules can also help rotate the spindle into a defined orientation relative to the cell axis (Giansanti et al., 2001; O'Connell and Wang, 2000; Palmer et al., 1992). The spindle determines the plane of cytokinesis, and spindle orientation thus has important consequences for the distribution of the cellular contents to the daughter cells, as well as the relative placement of the daughter cells within the tissue (i.e. side by side versus one on top of the other) (Giansanti et al., 2001; Glotzer, 2004; Rappaport, 1997). Spindle position and orientation play an important role in determining whether cell division is symmetric (producing identical daughter cells) or asymmetric (resulting in different daughter cell contents, placements or fates) (Betschinger and Knoblich, 2004; Neumüller and Knoblich, 2009). Appropriate daughter cell placement is crucial for tissue morphogenesis and homeostasis (Baena-López et al., 2005).

Cancer can arise from mutations in oncogenes, which act dominantly, or in tumor suppressor genes, for which loss of function is tumorigenic. Germline tumor suppressor mutations produce familial cancer syndromes with specific patterns of tumor development, and sporadic tumors often arise from somatic mutations of the same genes (Al-Sukhni et al., 2008; Dunbier and Guilford, 2001; Kim et al., 2010; Nakamura et al., 1991; Santoro et al., 1993). The tissue specificity of these mutations might be explained by their tissue-specific functions or by the occurrence of additional tissue-specific mutations. Whether spindle orientation is more important in some tissues compared with others is unknown.

Tumors of epithelial cells make up the vast majority of human cancers (Miller et al., 2005), and the tumor suppressors we discuss with roles in spindle orientation all prevent epithelial tumorigenesis (Gnarra et al., 1994; Hajra and Fearon, 2002; McCartney and Näthke, 2008; Nyhan et al., 2008; Senda et al., 2007). Our Commentary will therefore focus on spindle orientation mechanisms and consequences for epithelial tumors, although they might be relevant for other tumor types as well. We first present an overview of the spindle orientation process, followed by a description of the roles of the tumor suppressor mutations in spindle misorientation. We then speculate on the potential consequences of spindle misorientation for cancer development.

Patterns of spindle orientation

In most epithelia, the common pattern of spindle orientation is planar, in which the spindle is aligned along the tissue plane,

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parallel to the apical and basal surfaces of the cell (Fernández-Miñán et al., 2007; Fischer et al., 2006; Fleming et al., 2007; Lu et al., 2001) (Fig. 1). Planar spindle orientation leads to the establishment of a cytokinetic furrow that bisects the apical and basal cell surfaces, thereby generating daughter cells that are side by side in the tissue. Both daughter cells retain or re-establish contact with the extracellular matrix (ECM), and they attach to each other along their lateral surfaces (Jinguji and Ishikawa, 1992). Because daughter cells inherit identical contents and ECM attachments, planar spindle orientation can result in symmetric cell division (Fig. 2A). Loss of planar orientation in tumors could disrupt epithelial tissue morphology by placing daughter cells one on top of the other, creating vertical tissue expansion.

Although planar spindle orientation can result in symmetric cell division, it can also lead to asymmetric cell division. For example, in the developing mammalian brain, dividing cells can show planar spindle orientation, but partition cellular components – such as the apical surface and the basal process connecting it to the ECM, unequally – resulting in asymmetric cell division (Kosodo et al., 2004; Kosodo et al., 2008; Siller and Doe, 2009). Uneven distribution of microenvironmental factors, such as gradients of growth factors, could also influence the fates of daughter cells. This might be the case in intestinal crypts, where we found planar spindle orientation in dividing crypt cells, but as the crypt axis is itself polarized from base to apex, the daughter cell that is placed farther up the crypt–villus axis might be exposed to a different microenvironment that could affect crypt homeostasis (Fleming et al., 2007).

The other major pattern of spindle orientation is apico-basal, in which the spindle is aligned along the apico-basal cell axis, perpendicular to the tissue plane (Fig. 2B). The cytokinesis furrow in this case generates one daughter cell on top of the other. The apical daughter cell inherits the apical cytoplasm and cell cortex, and the basal daughter cell inherits the basal components. This asymmetric distribution of cell contents and daughter cell placement results in asymmetric cell division, and can generate divergent daughter cell fates (Cabernard and Doe, 2009). Apico-basal spindle orientation is important in developmental processes and in homeostasis within stem cell populations (Caussinus and Gonzalez, 2005; Deng and Lin, 1997; Gonzalez, 2007; Sousa-Nunes et al., 2010; Yamashita, 2009; Yamashita et al., 2003).

The tissue environment can affect spindle orientation. For example, in neural retina explants, most spindles show planar orientation, but the presence of the underlying retinal pigment epithelium biases this towards apico-basal orientation (Cayouette et al., 2001; Tibber et al., 2004). Another interesting example occurs in mammalian heart development, where epicardial lining cells show planar spindle orientation, which then converts to apico-basal orientation during an epithelial to mesenchymal transition (EMT, discussed later), in which these cells invade the myocardium to become fibroblasts and vascular smooth muscle cells (Wu et al., 2010). Shifts between planar and apico-basal orientation could impact the growth and differentiation of precancerous and tumor tissues, by altering the relative placement of daughter cells and the microenvironments to which individual tumor cells are exposed (Gonzalez, 2007; Morrison and Kimble, 2006; Neumüller and Knoblich, 2009).

The size of stem cell populations might also be controlled through spindle orientation. Stem cells have been observed to be capable of dividing symmetrically or asymmetrically in fly and mammalian neuroepithelia (Buchman and Tsai, 2007; Chenn and McConnell, 1995; Egger et al., 2007; Egger et al., 2010; Knoblich, 2008; Shen et al., 2002; Xie and Chin, 2008; Yamashita, 2009). Asymmetric division of stem cells could produce one replacement stem cell and one differentiating cell, which could maintain the overall size of the stem cell compartment (Fig. 2B). Fly germline stem cells are an example in which stem cell homeostasis is associated with asymmetric cell division (Yamashita, 2009; Yamashita et al., 2003). By contrast, mammalian skin stem cells divide symmetrically during development to increase the skin surface area and subsequently orient their spindles apico-basally to produce stratified skin (Lechler and Fuchs, 2005). Recently, two groups have presented evidence that symmetric cell division plays a fundamental role in intestinal stem cell populations (Lopez-Garcia et al., 2010; Snippert et al., 2010). On the basis of these observations, we expect that specific regulatory patterns will be found in the stem cell compartments of other tissues.

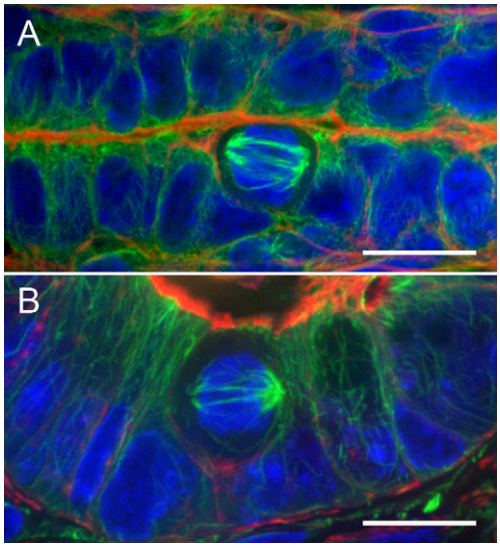

Fig. 1. Mammalian epithelial cells show planar spindle orientation. Confocal immunofluorescence images of (**A**) mouse mammary duct epithelium and (**B**) small intestinal crypt epithelium. Spindle microtubules are green, DNA is blue and polymerized actin is red. The apical cell surfaces that line the duct and crypt lumens are at the top of the frames. The dividing cells shown are in early anaphase, with the spindle located near the apical cell surface, and aligned parallel to the apical surface and hence to the tissue plane. Contrast was digitally altered to show all features of the image optimally. Scale bar: 10 μm.

The effect of spindle orientation on the size of a stem cell population is highly relevant to cancer, because many tumors are thought to contain cancer stem cells analogous to somatic stem cells, with a prominent hierarchical role in tumor composition (Alison et al., 2010; Mani et al., 2008). Mutation of the APC tumor suppressor in colon cancer stem cells results in rapid adenoma formation, whereas APC mutation in the more short-lived transit amplifying cell (TAC) compartment does not, suggesting that stem cells might be the cell of origin in colon cancer (Barker et al., 2009). We imagine that cancer stem cells can also divide asymmetrically or symmetrically, by analogy to non-cancerous stem cells. This idea is consistent with the observation that heterozygosity for APC mutation in intestinal stem cells alters the ratio between spindles with apico-basal orientation and those with planar orientation (discussed further in the section on APC) (Quyn et al., 2010). It is possible that tumor-associated spindle orientation

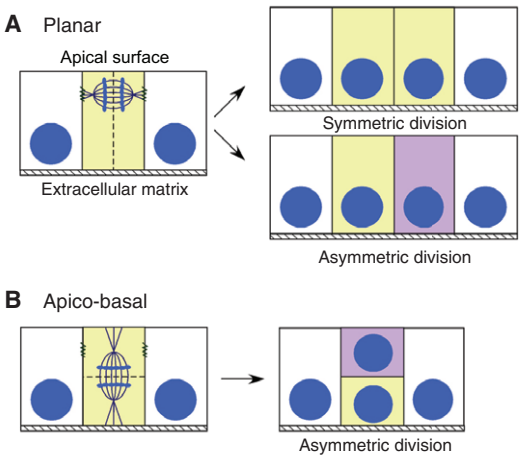

Fig. 2. Patterns of spindle orientation influence daughter cell placement within the tissue. (**A**) Planar orientation. The spindle is oriented parallel to the tissue axis. This produces a cell division plane that divides the cell vertically, resulting in daughter cells that are placed side by side along the ECM (shown as a hatched bar underneath the cells). Division of these cells can be symmetric or asymmetric, depending on the partitioning of cellular components. In a cancer stem cell population, planar spindle orientation with symmetric cell division could increase the number of cancer stem cells by producing identical daughter cells with cancer stem cell phenotypes. (**B**) Apico-basal orientation. The spindle is oriented perpendicular to the tissue axis. This produces a cell division plane that divides the cell into daughter cells one on top of the other, resulting in asymmetric division. Apico-basal spindle orientation would be expected to maintain the size of a cancer stem cell population by generating one replacement stem cell and one differentiating cell. Nuclei are blue; the colors of the cytoplasm represent components that differ between daughter cells.

defects could contribute to such a shift from asymmetric to symmetric cell division in cancer stem cells, thereby enhancing cancer stem cell-associated tumor growth, metastasis and recurrence (Neumüller and Knoblich, 2009).

Regulation of spindle orientation

The two patterns of spindle orientation described above depend on specific regulatory mechanisms. Primitive eukaryotes, such as yeast, flies and worms, have provided major insights into the proteins involved; many of these proteins were later confirmed to be present in vertebrates. Details of their roles have been extensively described in many excellent reviews (Cowan and Hyman, 2004; Knoblich, 1997; LaFlamme et al., 2008; Neumüller and Knoblich, 2009; Roegiers and Jan, 2004; Segalen and Bellaïche, 2009; Siller and Doe, 2009; Toyoshima and Nishida, 2007b; Wodarz, 2005). Here, our goal is to highlight two major components of the spindle orientation process that are likely to be altered in human tumors: the establishment of polarity with the associated generation of cortical cues, and the extension of astral microtubules to the cell cortex, which allows them to interact with cortical sites.

The establishment of epithelial polarity is associated with the formation of cell–cell junctions and cell–ECM interactions (Nelson et al., 1991; Vega-Salas et al., 1987). Thus, factors such as integrins at sites of ECM contact, junctional proteins at sites of cell–cell contact and diffusible molecules all have the potential to contribute to the generation of polarity cues that could be useful for orienting spindles.

A current model posits that, in polarized epithelial cells, adherens junctions (AJs) and, by implication, their associated proteins are the site of interaction between the cell cortex and astral microtubules, based on their location and the observation of microtubule plus ends ending in the AJ region (Bellett et al., 2009; Ligon and Holzbaur, 2007; Ligon et al., 2001; Meng et al., 2008) (Fig. 1). Whether the actual junction is required for generating the alignment cue is not clear. In cultured epithelial cells, ligation of the AJ protein E-cadherin on the basal cell surface results in partial spindle rotation towards this surface, suggesting that E-cadherin could play a major role in generating alignment cues (den Elzen et al., 2009). Further experiments using disruption of AJ-associated proteins and higher-resolution imaging techniques might be helpful in determining whether AJs are necessary and sufficient for planar spindle orientation. The disruption of AJs or the loss of associated cortical proteins might be a mechanism underlying spindle misorientation in cancer.

Astral microtubules link cortical cues to the spindle (Giansanti et al., 2001; O'Connell and Wang, 2000; Palmer et al., 1992). These microtubules must be able to extend to the cell cortex, which might be a considerable distance, depending on the geometry of the cell. Thus, their extension might require stabilizing factors, and loss of these stabilizing factors could be a potential mechanism of spindle misorientation in cancer (Hernandez and Tirnauer, 2010). The factors responsible for mediating microtubule stability and microtubule–cell cortex interactions in epithelial cells might include the tumor suppressors discussed in the following section and the force-generating motor protein cytoplasmic dynein (Faulkner et al., 2000; Green et al., 2005; O'Connell and Wang, 2000; Toyoshima and Nishida, 2007a).

Contributions of tumor suppressor mutations to spindle misorientation

Although misoriented spindles have been seen in several tumor models, not all tumors show spindle misorientation (Fleming et al., 2009). Rather, it seems that only certain mutations affect the spindle orientation process. Notably, these include mutations in the tumor suppressors APC, E-cadherin and VHL (Caldwell et al., 2007; den Elzen et al., 2009; Fleming et al., 2009; Quyn et al., 2010; Thoma et al., 2009). All three of these tumor suppressor proteins possess the dual functions of regulating epithelial polarity and stabilizing microtubules, perhaps suggesting biological connections between these two functions and highlighting their interplay in epithelial cells (Calzada et al., 2006; Piepenhagen and Nelson, 1998; Shi et al., 2004; Wollner and Krzeminski, 1992).

APC

In the familial adenomatous polyposis (FAP) syndrome, patients inherit a germline mutation of the *APC* tumor suppressor from one parent and develop colon cancer associated with loss of heterozygosity for the wild-type *APC* allele (Al-Sukhni et al., 2008; Fodde and Smits, 2001). The majority of sporadic colon cancers are also attributed to APC mutations (Kinzler and Vogelstein, 1996). Most APC mutations result in the expression of a truncated protein, although complete loss of APC is also highly tumorigenic (Cheung et al., 2010; Dihlmann et al., 1999). APC mutations impair the ability of APC to downregulate canonical Wnt/β-catenin signaling, which in turn increases cell proliferation and reduces apoptosis (for a review, see Bienz and Clevers, 2000). They also abolish binding sites for cytoskeletal proteins, which leads to decreased cell migration and reduced fidelity of

chromosome segregation (Fodde et al., 2001; Kaplan et al., 2001; Kawasaki et al., 2003; Kroboth et al., 2007; Mahmoud et al., 1997; Sansom et al., 2004; Wong et al., 1996).

In addition to these functions, APC is the best-studied tumor suppressor with regard to its role in spindle orientation. This role has been demonstrated in several model systems and in human cancers (Caldwell et al., 2007; Fleming et al., 2009; Lu et al., 2001; Quyn et al., 2010). Interestingly, the mechanisms used by APC to orient spindles appear to depend on the model system. APC knockdown by RNA interference (RNAi) or transfection of a dominant-negative APC fragment in HEK293 cells show dramatic spindle misorientation due to the complete destabilization of astral microtubules. This is mediated by the loss of the interaction between APC and its binding partner EB1, a microtubule-plus-end tracking protein (+tip) (Green et al., 2005; Schuyler and Pellman, 2001). This is consistent with several studies that demonstrate a role for APC in microtubule stabilization through its binding to microtubules and EB1 (Munemitsu et al., 1994; Nakamura et al., 2001; Smith et al., 1994; Wen et al., 2004; Zumbrunn et al., 2001).

Other studies have shown roles for APC in cell polarization, suggesting that its mutation could also cause spindle misorientation by altering cortical cues (Etienne-Manneville et al., 2005; Prosperi et al., 2009; Shi et al., 2004; Watanabe et al., 2004). These roles could be mediated by interactions between APC and components of the actin cytoskeleton or the AJ-associated protein β-catenin, as both of these interactions are lost with cancer-associated mutations (Nathke, 2005). In line with this role for APC, we found that heterozygous and homozygous APC mutation in mouse tissues causes spindle misorientation without eliminating astral microtubules (Fleming et al., 2009). The lack of an effect on astral microtubule length in vivo might be due to the inherently greater microtubule stability of polarized epithelial cells in intact tissues compared with less polarized cultured cells (Musch, 2004). Thus, APC might control spindle orientation through effects on astral microtubule stabilization, cell cortex polarization, or both.

Beyond this general role for APC in spindle orientation, a specific role for APC in regulating spindle orientation in stem cells has recently generated interest (Quyn et al., 2010). This study analyzed spindle orientation in distinct crypt compartments (stem cell versus TAC) in premalignant tissues that are heterozygous for an APC mutation (Quyn et al., 2010). In wild-type animals, most TACs have planar spindle orientation, whereas the majority of stem cells show apico-basal orientation (Quyn et al., 2010), although the apparent differences between this result and those suggested by other groups have not been completely reconciled (Lopez-Garcia et al., 2010; Snippert et al., 2010). In the study by Quyn et al., stem cells from mice and human patients that are heterozygous for APC mutations show planar spindle orientation (Quyn et al., 2010). This switch from apico-basal to planar spindle orientation in intestinal stem cells could be responsible for increasing the pool of premalignant stem cells that specifically harbor the APC mutation. Further studies of the role of APC in intestinal stem cell spindle orientation and its alterations in intestinal cancer should prove informative.

E-cadherin

Patients with a germline E-cadherin mutation develop familial diffuse gastric cancer (FDGC), a syndrome that includes gastric and lobular breast cancers (Dunbier and Guilford, 2001). These tumors show major reductions in cell–cell attachment and metastasize early (Humar and Guilford, 2009). Sporadic lobular breast cancer and its precursor lesion lobular carcinoma in situ (LCIS), as well as many other sporadic tumor types, also show reduced or absent E-cadherin expression (Hajra and Fearon, 2002; Strathdee, 2002; Vos et al., 1997). E-cadherin is the major component of AJs in epithelial cells and is thus a major controller of the formation of epithelial cell–cell attachments (Capaldo and Macara, 2007). E-cadherin mutation might release its binding partner β-catenin from AJs, leading to cancer-promoting effects on gene transcription, apoptosis signaling and angiogenesis (Ceteci et al., 2007; Derksen et al., 2006; Onder et al., 2008).

In addition to its tumor suppressor function, E-cadherin plays a major role in preventing tumor metastasis. Loss of E-cadherin has been noted at the edge of tumors, suggesting a relationship between its loss and cell egress from the tumor front (Brabletz et al., 2001; Hsu et al., 2007; Wang et al., 2009). Forced expression of E-cadherin prevents invasive phenotypes in vitro and is able to block the transition from adenoma to invasive carcinoma in a mouse model of pancreatic cancer (Perl et al., 1998; Vleminckx et al., 1991). Suppression of E-cadherin by RNAi, but not merely the loss of cell–cell adhesion, increases metastasis, demonstrating that the metastasis-preventing role of E-cadherin goes beyond that of AJ formation (Onder et al., 2008). This further role for E-cadherin might be its ability to block aspects of the EMT, a phenotypic transformation in which non-motile cells adopt a fibroblastic morphology and increase their motility, migration and invasion capacity (for reviews, see Lombaerts et al., 2006; Thiery et al., 2009; Yang et al., 2004). EMT is associated with reduced expression of E-cadherin and increased expression of N-cadherin, which, despite its overall similarity to E-cadherin, is unable to support the same functions (such as the strength of cell–cell attachments) (Chu et al., 2005; Nieman et al., 1999; Rieger-Christ et al., 2004).

E-cadherin has been recently shown to play a role in spindle orientation in polarized epithelial cells (den Elzen et al., 2009). Here, reduction of E-cadherin by either RNAi or expression of a dominant-negative version of the protein impairs spindle orientation (den Elzen et al., 2009). As discussed for APC, potential mechanisms by which E-cadherin mutation could misorient spindles include reduced cell polarization and destabilization of astral microtubules (through reduced microtubule interactions with cell junctions or an EMT) (Chausovsky et al., 2000; Dugina et al., 1995; Shtutman et al., 2008; Stehbens et al., 2009). A third possible mechanism of spindle misorientation seems to be loss of cortically localized APC in cells subjected to E-cadherin RNAi, suggesting a possible shared role for APC and E-cadherin in spindle orientation (den Elzen et al., 2009).

VHL

Patients who inherit a germline mutation of the VHL tumor suppressor develop von Hippel-Lindau syndrome, characterized by benign cysts and malignant tumors in several organs, perhaps highlighting distinct roles for VHL in tissue morphogenesis and tumorigenesis (for reviews, see Friedrich, 1999; Maher and Kaelin, 1997). The best-appreciated tumor suppressor function of VHL is its role as a key component of an E3 ubiquitin ligase that targets hypoxia-inducible factor (HIF) for degradation, thus controlling the response of a cell to local oxygen tension (Epstein et al., 2001; Ivan et al., 2001; Jaakkola et al., 2001; Kaelin, 2008; Nyhan et al., 2008). Reduced HIF degradation and loss of HIF-independent functions of VHL increase cell proliferation and angiogenesis, prevent cell death and reduce cell–cell adhesion (Kaelin, 2008; Krishnamachary et al., 2006; Nyhan et al., 2008; Ohh, 2006).

VHL also plays a role in spindle orientation (Thoma et al., 2009). VHL RNAi in HeLa cells causes spindle misorientation by eliminating astral microtubules, similar to findings with APC in cultured cells (Green et al., 2005; Thoma et al., 2009), and is consistent with the known contribution of VHL to microtubule stability (Hergovich et al., 2003; Hergovich et al., 2006; Lolkema et al., 2007). However, as HeLa cells are tumor-derived cells that fail to form AJs, it is not known whether loss of VHL function causes a similar defect in vivo or whether the roles of VHL in cell polarity might be of greater importance (Stöffler et al., 1998). VHL also mediates expression of E-cadherin in some systems and its mutation could thus contribute to spindle misorientation by reducing E-cadherin levels (Esteban et al., 2006; Evans et al., 2007; Krishnamachary et al., 2006). The VHL target HIF1 was also recently shown to repress APC expression, suggesting that reduction of APC levels might be another potential mechanism by which VHL mutation could cause spindle misorientation and potentially linking all three of these tumor suppressors in the spindle orientation process (Newton et al., 2010).

Other mechanisms of spindle misorientation in cancer

There are several other cancer-associated changes that might also result in spindle misorientation, but for which there are less experimental data on human cancers. These include mutations of oncogenes, tumorigenic mutations in flies that affect centrosome number, and alterations in the microenvironment that might affect the spindle orientation process, as described below.

Mutations of oncogenes

Unlike tumor suppressor inactivation, a clear link between oncogene activation and spindle misorientation is lacking. Some oncogenic signaling pathways have been implicated in the spindle orientation process, but rather than promoting spindle misorientation, they seem to be required for appropriate spindle orientation. For example, phosphoinositide 3-kinases (PI3Ks), which are activated by growth factor stimulation, are required for appropriate spindle orientation that is mediated by integrin engagement in HeLa cells (Toyoshima et al., 2007). This is notable because inhibition of PI3K signaling is being considered as a cancer treatment strategy (Courtney et al., 2010). Blocking oncogenic activation of PI3K might cause spindle misorientation as a side effect, although it is not clear whether the role of PI3K in spindle orientation would also be seen in polarized epithelial cells in vivo (Toyoshima et al., 2007).

Mutations that affect centrosome function and cell polarity in flies

Another intriguing connection between tumorigenesis and spindle misorientation is seen with mutations that induce centrosome amplification (Basto et al., 2008; Castellanos et al., 2008). Several mutations that increase centrosome number in flies are found to produce extensive growth and metastasis of tumor implants, along with prominent spindle misorientation (Basto et al., 2008; Castellanos et al., 2008). Centrosome amplification is a feature of many human tumors, but most studies in mammalian systems have focused on its contribution to the development of aneuploidy rather than to spindle misorientation (Fukasawa et al., 1996; Lingle et al., 1998; Mayer et al., 2003; Tutt et al., 1999). It is not clear which factors determine whether centrosome abnormalities predominantly cause chromosome missegregation, spindle misorientation or both, and whether these differences are species-dependent. It will be interesting to see whether homologs of these genes are mutated in human tumors and, if so, whether their mutation is associated with spindle misorientation.

Alterations in the microenvironment

In addition to gene mutations, the role of the microenvironment, which includes infiltrating cells, secreted growth factors and altered matrix components, is being increasingly recognized in tumor biology (for reviews, see Calorini and Bianchini, 2010; Chiodoni et al., 2010; McAllister and Weinberg, 2010). We imagine that spindle orientation could be sensitive to microenvironmental changes. For example, spindle axis is altered in polarized Madin-Darby canine kidney (MDCK) cells that are exposed to hepatocyte growth factor (HGF, also known as scatter factor), which is overexpressed in some tumors and is implicated in the cancer-associated EMT (Behrens et al., 1991; Savagner et al., 1997; Yu et al., 2003). Altered ECM composition or stiffness, and the resulting changes in integrin expression, are another example of a microenvironmental change that could affect spindle orientation, as integrin engagement regulates spindle orientation in cultured cells (Toyoshima and Nishida, 2007a). A future challenge will be to determine whether the tumor microenvironment affects spindle orientation in vivo and, if so, whether these effects can be manipulated pharmacologically.

The role of spindle misorientation in cancer – correlation, causation or contribution?

Some of the abnormalities seen in tumors merely reflect phenotypic changes that play no causative role in the tumor, but in the case of spindle misorientation, we interpret the specific association of this phenomenon with the mutations described above to suggest that spindle misorientation does contribute to cancer in some way. Does spindle misorientation alone cause cancer? Or is it more likely to contribute to the evolution of aggressive cancer phenotypes in conjunction with conventional changes in tumor suppressor genes?

Testing a direct tumorigenic role for spindle misorientation is difficult, as tools to disrupt spindle orientation without affecting other spindle functions are lacking. For example, manipulations that affect astral microtubules, such as mutation of APC or treatment with a microtubule-destabilizing drug, are likely to affect both astral and kinetochore microtubules, and hence are likely to impair spindle orientation and chromosome segregation (Green et al., 2005). The tumor suppressors mentioned above also have crucial functions in limiting cell growth and survival, making it difficult to separate the contribution of spindle misorientation from these other effects. True separation-of-function alleles of the tumor suppressors that control spindle orientation and the genes involved in regulating centrosome number would be extremely useful in this regard.

In the meantime, we believe there is reasonable evidence suggesting that spindle misorientation alone is not tumorigenic. This is based on the finding that mice with polycystic kidney disease caused by germline deletion of the transcription factor hepatocyte nuclear factor 1 β (HNF1β) or the ciliary kinesin KIF3a show spindle misorientation and associated morphological changes in affected kidneys, but these animals are not tumor prone (Fischer et al., 2006; Patel et al., 2008). Other mutations that result in polycystic kidney disease and loss of planar cell polarity do not show increased cancer rates, although spindle orientation has not been specifically assayed in these cases (Bergmann et al., 2008;

Ross et al., 2005; Yates et al., 2010). These studies suggest that spindle misorientation might affect tissue morphology during development, but does not cause tumors. Rather, we believe that spindle misorientation has the potential to play a substantial synergistic role with other changes that occur at various stages of tumor development and progression. Below, we discuss three possible means by which spindle misorientation could affect tumor evolution: increasing aneuploidy, facilitating tissue disorganization and metastasis, and expanding the cancer stem cell pool.

Increasing aneuploidy

Aneuploidy (abnormal chromosome number) is a major component of many human tumors (Chandhok and Pellman, 2009; Weaver and Cleveland, 2006). Although there might not be a simple answer as to whether aneuploidy causes cancer, a preponderance of evidence suggests that it can accelerate genetic changes, which leads to cancer in many settings (Chandhok and Pellman, 2009; Hahn and Weinberg, 2002; Hanahan and Weinberg, 2000).

Spindle misorientation and aneuploidy are connected in two ways. First, spindle orientation and appropriate chromosome segregation both require the end-on interactions of a subset of microtubules with other cellular structures, although the permanence or transience of these attachments might differ significantly. Many of the same microtubule plus-end tracking proteins (+TIPS) and their associated factors that affect spindle orientation (such as EB1 and APC) also affect the fidelity of chromosome segregation (Green et al., 2005; Vallee et al., 2001). Thus, alteration of these microtubule plus end regulators in cancer would be expected to cause aneuploidy and spindle misorientation simultaneously.

Second, spindle misorientation might worsen or even facilitate the development of aneuploidy through its effects on cytokinesis (Fig. 3B). This has been demonstrated for APC in experiments in which spindle misorientation induced by RNAi of APC also causes a failure of cytokinesis, resulting in tetraploidy (Caldwell et al., 2007). Tetraploidization is also detected in tissues of APC mutant mice and could set the stage for further genetic changes (Caldwell et al., 2007). The contribution of these two mitotic abnormalities to cancer is likely to be further amplified by the rapid cell proliferation seen in most tumors, through increases in the number of mitotic events and decreases in the time available to correct mitotic errors.

Disorganization of tissue morphology and promotion of metastasis

As mentioned earlier, planar spindle orientation results in both of the daughter cells maintaining proximity or contact with the ECM. By contrast, when the spindle is misoriented, one daughter cell could physically separate its sister from the ECM. This might lead to several possible outcomes (Fig. 3C). The unattached daughter cell could die as a result of extrusion and loss of anchorage, but the loss of appropriate apoptotic mechanisms typical of cancer cells might prevent this (Slattum et al., 2009). Alternatively, it could adhere to the cell below it, resulting in vertical tissue expansion and tissue hyperplasia, which is considered a premalignant change (Fitzgibbons et al., 1998; Jones and Young, 1994; Tobi, 1999). Finally, if the tissue geometry favored the creation of a lumen, it might result in the formation of a new gland. Although this has not been shown in vivo, studies using three-dimensional culture show that spindle misorientation correlates with altered epithelial organization, such as multiple lumen formation (Jaffe et al., 2008; Qin et al., 2010; Zheng et al., 2010). Thus, tissue morphology could be dramatically altered as a result of spindle misorientation and in a way that promotes the propagation of cells with abnormal genomes.

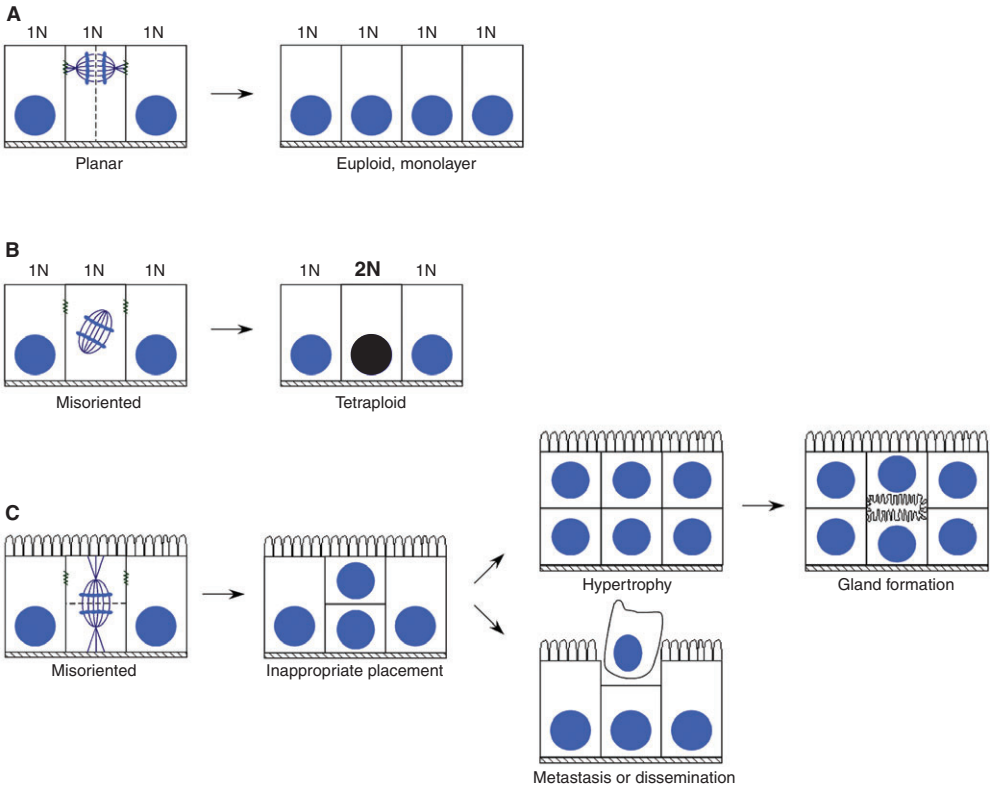

Fig. 3. Potential effects of spindle misorientation on the organization of a precancerous tissue or tumor. (**A**) Appropriate planar spindle orientation in an epithelium would produce euploid daughter cells in a simple monolayer. (**B**) Aneuploidy. Spindle misorientation associated with loss of astral microtubules could cause failure of cytokinesis, which would prevent cell division and lead to tetraploidization. The resulting single tetraploid daughter cell (black nucleus with twice the appropriate number of chromosomes, designated 2N) might more easily acquire further genetic changes that promote tumorigenesis. (**C**) Tissue disorganization and metastasis. Loss of planar spindle orientation in an epithelium could result in daughter cells being placed inappropriately one on top of the other. Several possible outcomes could include tissue hypertrophy, new gland formation (with generation of a new lumen) and shedding of the apically placed daughter cell, which could contribute to dissemination in fluid compartments, such as ascites and effusions. The scalloped section on the apical surface of the cells represents the brush border.

Spindle misorientation has also been proposed to facilitate metastasis. In a cell culture study in which spindle misorientation is induced by overexpression of the Rho GTPase, daughter cells that lack ECM contact float away in the culture media without loss of viability and re-adhere to the dish at another site (Vasiliev et al., 2004). Although this system fails to recapitulate many aspects of lymphovascular metastasis from solid tumors, it might reflect some aspects of metastasis, such as loss of cell–cell contacts, and might cause tumor dissemination in fluid compartments, such as tumor-associated ascites or effusions (Fig. 3C). Alternatively, basal protrusion of a daughter cell could lead to metastasis (Slattum et al., 2009). Mutations that couple spindle misorientation with aspects of an EMT could be especially potent in promoting metastasis.

The relationship between tissue disorganization and spindle misorientation could also be bidirectional. Just as spindle misorientation might lead to tissue disorganization, the loss of normal polarity cues in disorganized tissues could result in further spindle misorientation. On the basis of our previous studies, we suspect that tumor formation by itself is insufficient to misorient spindles (Fleming et al., 2009). However, tumors with certain mutations or altered microenvironments might develop misoriented spindles along with other defects, resulting in a feedback loop between spindle misorientation and an altered microenvironment in premalignant tissues and tumors.

Expansion of the cancer stem cell compartment.

Cancer stem cells seem to play important roles in metastasis and drug resistance, and the number of cancer stem cells in a tumor might thus influence its aggressiveness and treatability (Alison et al., 2010; Rosen and Jordan, 2009). As noted earlier, heterozygosity for an APC mutation results in the loss of apico-basal spindle orientation in intestinal stem cells (Quyn et al., 2010), raising the possibility that spindle misorientation could alter the balance between asymmetric and symmetric division of cancer stem cells (Fig. 2).

To date, most of the experiments that address the role of spindle orientation in increasing the number of cancer stem cells have been done in flies. These show that alteration of genes involved in spindle orientation in larval neuroblasts [a type of progenitor cell (Knoblich, 2008)] causes tumor development and aneuploidy. This highlights potentially overlapping roles for these genes in multiple spindle functions and raises the possibility that spindle misorientation in stem (or progenitor) cells could contribute to tumor development and expansion (Betschinger et al., 2006; Caussinus and Gonzalez, 2005; Lee et al., 2006). These findings are of significant interest as they underscore the possibility that spindle misorientation in mammalian precancerous or cancerous stem cells could similarly increase the proportion of cancer stem cells in a human tumor. The occurrence of spindle misorientation in cancer stem cells, its impact on symmetric versus asymmetric cell division, and its role in tumorigenesis and progression are key questions for the future.

Conclusions and perspectives

Spindle orientation is a feature of three-dimensional tissue growth that profoundly affects tissue geometry, morphogenesis and function. Like the composition of the ECM or other features of the microenvironment, it alone is probably insufficient to initiate tumorigenesis, but its contribution to tumor development and behavior should not be ignored. Future studies of the mechanisms of spindle misorientation in cancer might clarify how tumor-associated mutations regulate this process, and whether they act independently or through a common pathway. It will be particularly interesting to see whether the roles of tumor suppressors and genes that control centrosome duplication in spindle orientation are separable from their other functions, including that of preventing aneuploidy.

Despite the complexity of spindle orientation regulation, it might be possible to correct spindle misorientation in cells of some premalignant tissues or tumors. Strategies to do so might include those that restore cell–cell junctions, cell polarity and microtubule stability in these cells. Correcting spindle orientation abnormalities could lower rates of aneuploidy, improve tissue organization, reduce metastasis and limit the proportion of cancer stem cells in a tumor, all worthy targets for biologically based therapies.

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