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Spindle Microtubule Dysfunction and Cancer Predisposition

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Chromosome segregation and spindle microtubule dynamics are strictly coordinated during cell division in order to preserve genomic integrity. Alterations in the genome that affect microtubule stability and spindle assembly during mitosis may contribute to genomic instability and cancer predisposition, but directly testing this potential link poses a significant challenge. Germ-line mutations in tumor suppressor genes that predispose patients to cancer and alter spindle microtubule dynamics offer unique opportunities to investigate the relationship between spindle dysfunction and carcinogenesis. Mutations in two such tumor suppressors, adenomatous polyposis coli (APC) and Shwachman–Bodian–Diamond syndrome (SBDS), affect multifunctional proteins that have been well characterized for their roles in Wnt signaling and interphase ribosome assembly, respectively. Less understood, however, is how their shared involvement in stabilizing the microtubules that comprise the mitotic spindle contributes to cancer predisposition. Here, we briefly discuss the potential for mutations in APC and SBDS as informative tools for studying the impact of mitotic spindle dysfunction on cellular transformation.
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Chromosome segregation during cell division is tightly regulated in order to preserve genomic integrity. To facilitate DNA segregation, cells assemble a microtubule-based machine, called the mitotic spindle, to capture, bi-orient and align chromosomes (McIntosh et al., 2002). Chromosomes make stable attachments to the tips of microtubules through specialized protein complexes called kinetochores. In order to properly attach to the mitotic spindle, chromosome pairs must bi-orient, with the kinetochores on replicated sister chromosome pairs attached to microtubules emanating from opposite spindle poles (Fig. 1). During chromosome attachment, erroneous connections can occur that result in the linkage of one kinetochore to both spindle poles, referred to as merotelic attachments (Fig. 1). Merotelic attachments can be promoted by abnormal spindle geometry or increased stability of kinetochore-microtubule attachments and the failure to resolve them leads to chromosome instability (Bakhoum et al., 2009a,b; Ganem et al., 2009; Silkworth et al., 2009) (Fig. 1). Both spindle formation and kinetochore-microtubule attachments rely on proper regulation of spindle microtubule dynamics, and cultured cells display a relatively low tolerance to alterations in these dynamics (Bakhoum and Compton, 2012b). Furthermore, the expression of many proteins with known roles in regulating microtubule dynamics during cell division is abnormal in chromosomally unstable cancer cells (Bakhoum and Compton, 2012a). These observations support the intriguing possibility that changes in mitotic spindle microtubule dynamics contribute to cancer development in humans. However, directly testing this idea is not trivial. In this article, we briefly discuss the utility of patient-derived mutations responsible for two genetically inherited cancer predisposition syndromes in studying the putative link between spindle microtubule dynamics and chromosome instability in cancer.

Altered Spindle Microtubule Dynamics May Contribute to Cancer Predisposition

Inherited cancer predisposition syndromes offer novel insights into the mechanisms underlying tumorigenesis in the general population. The disorders familial adenomatous polyposis (FAP) and Shwachman–Diamond Syndrome (SDS) are primarily caused by inactivating mutations in the adenomatous polyposis coli (APC) and Shwachman–Bodian–Diamond Syndrome (SBDS) genes, respectively (Groden et al., 1991; Kinzler et al., 1991; Boocock et al., 2003). SDS is an autosomal recessively inherited bone marrow failure syndrome

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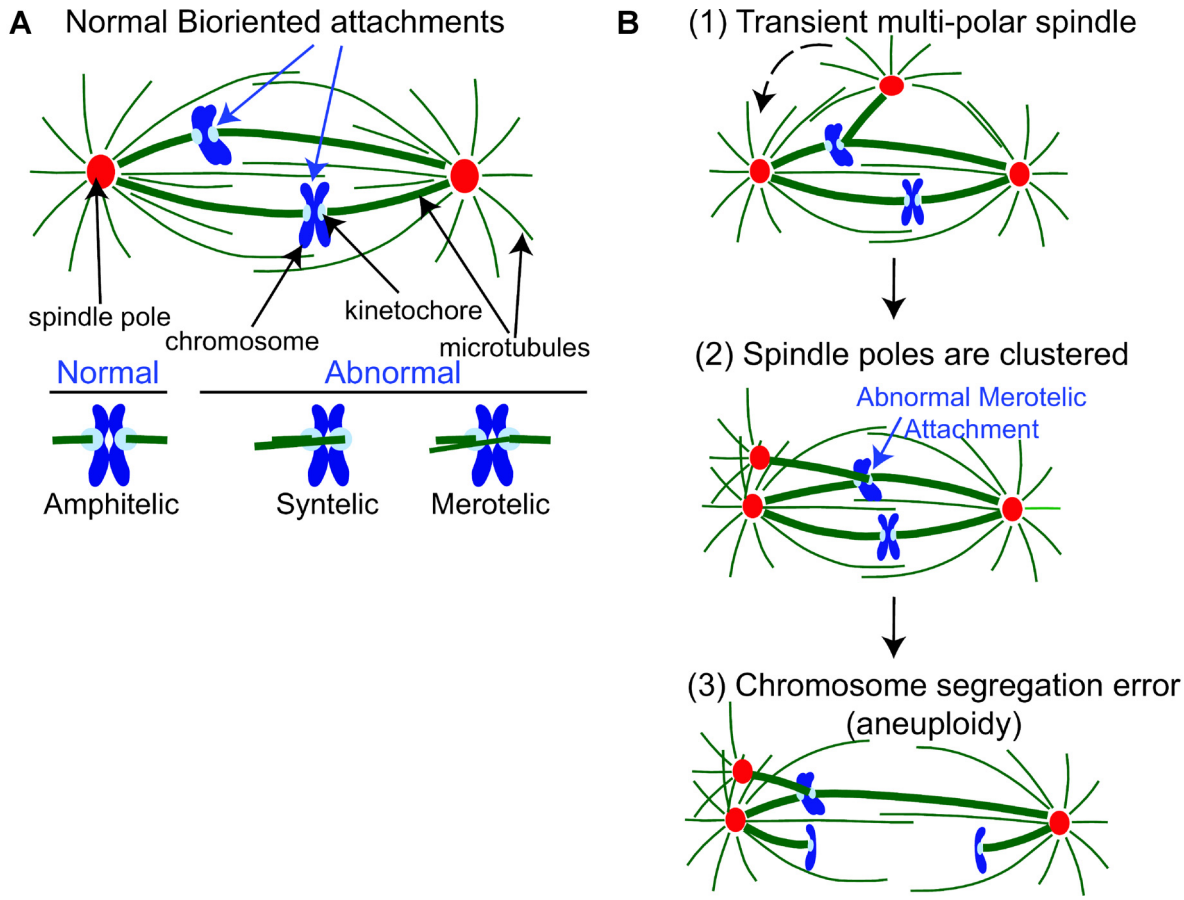

Fig. 1. Schematic of a mitotic spindle and kinetochore-microtubule attachment configurations. (A) Chromosomes achieve normal bioriented attachments to spindle microtubules when each sister kinetochore is connected to bundles of microtubules emanating from opposite spindle poles. However, errors in the attachment process can lead to the formation of syntelic and merotelic connections. (B) Abnormal microtubule stability or centrosome duplication can lead to the formation of transient multi-polar spindles that promote merotelic attachments and chromosome segregation errors.

associated with an increased incidence of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) (reviewed in [Dror, 2005; Shimamura, 2006]). Leukemias arising in these patients typically exhibit complex chromosomal abnormalities and aneuploidy. In contrast, FAP is inherited in an autosomal dominant pattern. It is characterized by the development of hundreds or thousands of benign polyps in the colon at an early age, which will become malignant during the adult years if the colon is not removed (Aretz, 2010; Jasperson et al., 2010). While the products of APC and SBDS are multifunctional and loss of their activity affects different tissues, both proteins function to stabilize spindle microtubules during cell division (Munemitsu et al., 1994; Smith et al., 1994; Kaplan et al., 2001; Tighe et al., 2004; Austin et al., 2008). Thus, altered spindle microtubule dynamics could contribute to cancer risk in SDS and FAP patients.

SBDS Mutations Impair Spindle Assembly and Chromosome Segregation Accuracy

SBDS has a well-studied function in ribosome assembly, but is also necessary for proper mitotic spindle assembly. In culture, primary cells derived from bone marrow of SDS patients display an increased incidence of multipolar spindles, which can result from unstable spindle microtubules. Consistent with a role for SBDS in stabilizing microtubules, cells from SDS patients are strikingly hypersensitive to the microtubule-destabilizing agent nocodazole and resistant to the microtubule-stabilizing agent paclitaxel. Furthermore, purified SBDS protein can directly stabilize microtubules in vitro (Austin et al., 2008). Loss of this stabilizing activity could explain the spindle defects observed in cells that lack SBDS, suggesting that unstable microtubules are one of the primary defects responsible for the chromosome instability observed in cells from SDS patients. These data support a model in which SBDS stabilizes microtubules within the mitotic spindle to promote its assembly and prevent genomic instability. Future studies aimed at understanding precisely how SBDS regulates microtubule stability will be necessary to understand the contribution of its mitotic function to leukemia predisposition and bone marrow failure.

Mutations in the C-Terminus of APC are Key for Mitotic Function and Correlate to Disease Severity

APC has reported roles in a diverse set of cellular processes, including a well-characterized function in the Wnt signaling pathway (Nelson and Nathke, 2013). However, like SBDS, it also functions to increase the stability of mitotic spindle microtubules and loss of this activity leads to spindle

orientation and chromosome segregation defects (Green and Kaplan, 2003; Green et al., 2005; Caldwell et al., 2007). Most APC mutations in patients with FAP truncate the protein's C-terminus. This region is required for its interaction with microtubules and mitotic functions (Kaplan et al., 2001; Tighe et al., 2004). Reinforcing the importance of the APC tumor suppressor, approximately 80% of sporadic colon cancer tumors have mutations in APC and 85% display chromosomal instability (Polakis, 1997). Most APC mutations cause "classical FAP" (Friedl and Aretz, 2005), however, some APC mutations truncate the protein after codon 1581, which primarily removes the microtubule binding domain of APC. These truncation mutations are associated with fewer polyps, a condition called attenuated FAP (AFAP) (Friedl and Aretz, 2005). This implies that loss of spindle function may be sufficient for polyp formation, and such loss synergizes with other APC functions (such as Wnt signaling) to determine the severity of polyposis and other clinical features. Thus, APC mutations present in patients with both classical and attenuated FAP offer potentially useful tools for determining the contribution of altered spindle microtubule dynamics to cancer development.

Investigating the Consequences of Defective Spindle Function in FAP and SDS

Cells with compromised APC or SBDS function are able to complete cell division but are prone to making chromosome segregation mistakes. It is not entirely clear what the downstream consequences of these defects are or how spindle destabilization contributes to cancer predisposition in FAP and SDS. Patient-derived mutations in the APC and SBDS tumor suppressor genes offer clues that may prove useful in studying the contribution of mitotic spindle dysfunction to oncogenesis. For example, how do reduced microtubule stability and chromosome segregation errors affect chromosome structure and function during interphase? Can measurable changes be detected in interphase nuclear topology when cells express disease-causing mutations? Investigations into the effects of mitosis-disrupting mutations in APC or SBDS on nuclear architecture could provide important information in addressing these fundamental questions.

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