heterozygous animals, which normally develop sarcomas at a higher incidence than lymphomas, *bub1* partial loss of function had no effect on sarcoma incidence. Though $APC^{min/+}$ colon tumor incidence was similarly increased in the $bub1^{+/-}$ setting, no change was seen in the incidence of small intestinal tumors, which are also seen in this strain. The mechanistic basis for this context dependence will require further studies. Technologies to carefully measure rates of aneuploidy and LOH in early neoplastic lesions will undoubtedly aid in this analysis.

Previous studies have already suggested that CIN might, in some contexts, act as a tumor suppressor (Garcia-Higuera et al., 2008; Rao et al., 2005; Weaver et al., 2007). Baker et al.'s study reinforces the notion that whether CIN favors or prevents tumor formation and progression is very much subject to the setting under which it occurs. Certain cell types may be particularly sensitive to abnormal chromosome complements and be eliminated in early lesions. Whether non-cell-autonomous effects are at play in such suppression has not yet been analyzed. Aneuploidy in cells of the tumor microenvironment, for example, may contribute in some tissue types to tumor suppression. Conditional models of CIN will be required to address this issue.

The finding that, in the setting of *p53* or APC^{min} heterozygosity, the mutant allele is similarly duplicated strongly suggests that there is selective pressure to maintain two copies of the chromosome harboring the tumor suppressor that undergoes LOH. Haploinsufficiency of genes on this chromosome likely decreases the fitness of cells that have undergone such a loss, and these cells are rapidly outcompeted in the population.

The results presented in this issue by Baker et al. provide the first evidence for a potential mechanism by which CIN can lead to tumorigenesis by linking aneuploidy to loss of heterozygosity of tumor suppressor genes. As so often happens with important advances in cancer biology, more questions are now raised. Could CIN favor the duplication of chromosomes harboring oncogenic mutations at the same time as it accelerates the loss of tumor suppressors? Does CIN occur prior to the loss of tumor suppressors and is it generally present in early preneoplastic lesions as one might predict from the current analysis? Does such a mechanism of mutant chromosome retention occur in human cancers?

Nonetheless, this study uncovers an important mechanistic insight into how LOH is accelerated by defects in the mitotic checkpoint pathway and paves the way for a deeper understanding of the role of CIN in cancer initiation and progression.

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Proliferation and Tumor Suppression: Not Mutually Exclusive for Eph Receptors

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Eph receptors are important but controversial regulators of cancer development. A recent study reported in *Cell* reveals that in the intestinal epithelium, EphB2 enhances proliferation through a kinase-dependent pathway and inhibits migration independent of its kinase activity. These separate pathways simultaneously promote proliferation but suppress invasive growth of intestinal adenomas.

Extensive evidence implicates the Eph receptor family of tyrosine kinases in cancer development, but it remains incompletely understood how these receptors affect cancer progression. Opposite tumor-promoting and tumor-suppressing effects have been described, sometimes for the same Eph receptor in the same type of cancer (Pasquale, 2008). Switching

between opposite activities has been attributed to contextual factors, such as activation of Eph-dependent tumor suppressor pathways by ephrin ligands versus hijacking of Eph receptors by oncogenic signaling pathways.

Eph receptors of the B class, which bind the transmembrane ephrin-B ligands, have been well studied in the intestine. The EphB2, EphB3, and EphB4 receptors and the ephrin-B1 and ephrin-B2 ligands are expressed in complementary gradients along the crypts under the control of the Wnt/β-catenin/Tcf pathway, which upregulates EphB and downregulates ephrin-B expression (Batlle et al., 2002). Thus, EphB receptors are expressed in the proliferating progenitor cells located near the bottom of the crypts, close to the source of Wnt protein. As progenitor cells migrate toward the intestinal lumen, they gradually lose EphB and acquire ephrin-B expression while they differentiate before being shed. EphB repulsive signaling determines progenitor cell positioning by preventing premature migration into the more differentiated ephrin-B regions. Illustrating the complexity of Eph functions, EphB signaling also promotes proliferation, which is a less common Eph activity and occurs independently of nuclear β-catenin (Figure 1; Holmberg et al., 2006).

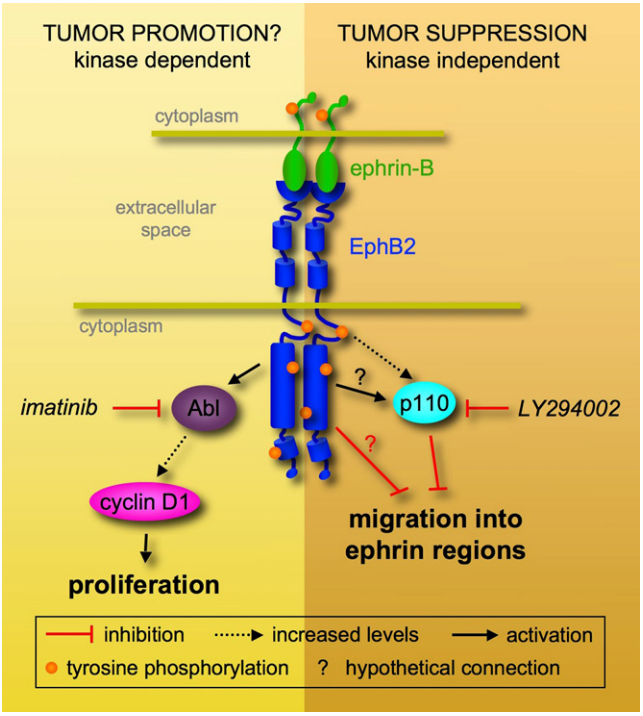

Figure 1. EphB2 Promotes Proliferation and Inhibits Migration in Intestinal Cells through Distinct Pathways

(Left) EphB2 interaction with ephrin-B ligands in the normal intestine increases cyclin D1 protein levels through Abl, thereby promoting cell proliferation. In intestinal adenomas, EphB2 is overexpressed and also promotes proliferation. The Abl inhibitor imatinib can block these effects.

(Right) EphB2 interaction with ephrin-B ligands upregulates transcripts encoding the p110α isoform of PI3 kinase and inhibits migration in a kinase-independent manner. The PI3 kinase inhibitor LY294002 blocks these effects. It is not known whether EphB2 may also stabilize p110 isoforms at the posttranscriptional level and/or stimulate PI3 kinase activity. Other pathways may also cooperate with PI3 kinase in EphB2-dependent repulsion. In adenomas, EphB receptors suppress tumor progression by preventing expansion into regions that express ephrin-B ligands.

These dual EphB activities play an important role in intestinal homeostasis and tumorigenesis. In the transition from normal cells to intestinal adenoma, EphB receptors are usually upregulated and ephrin-Bs downregulated by the constitutive activation of the β-catenin/Tcf pathway (Batlle et al., 2002). EphB receptors are responsible for about half of the proliferative activity in adenomas (Genander et al., 2009; Holmberg et al., 2006), but adenoma growth is restricted by repulsion from ephrin-Bs in the surrounding differentiated epithelium (Batlle et al., 2005; Cortina et al., 2007). The EphB proliferative effects may nevertheless have some tumor-promoting ability, as perhaps suggested by the decreased tumor counts in the small intestine of $APC^{min/+}$ mice with impaired EphB signaling (Batlle et al., 2005; Cortina et al., 2007). Although the β-catenin/Tcf pathway remains active in the more malignant colorectal carcinomas, EphB receptors are lost in many of the tumor cells, enabling invasiveness as well as tumor expansion through EphB-independent proliferation. This represents a critical step in the progression to malignant stages and correlates with a poor prognosis. Thus, EphB receptors promote proliferation but suppress adenoma growth.

Frisen and colleagues now shed light on this apparent paradox (Genander et al., 2009). They used a microarray approach to achieve a global view of the transcriptional changes occurring in the colon of mice injected with ephrin-B2-Fc, which promiscuously binds all EphB receptors. In this in vivo setting, ephrin-B2-Fc appears to function as an EphB antagonist (Holmberg et al., 2006), even though in other settings it can act as an agonist (Noren et al., 2006). Consistent with the functional data, the microarray analyses reveal that EphB receptors regulate genes involved in both cell proliferation and migration.

The authors resorted to in vivo analysis of mice receiving ephrin-B2-Fc as well as a series of mutant mice to dissect EphB signaling pathways in the intestine (Genander et al., 2009; Holmberg et al., 2006). Because previous analysis of EphB2 and EphB3 knockout mice had shown that the two receptors have partially redundant functions in intestinal cells (Batlle et al., 2002), various EphB2 mutants with increased or deficient kinase activity were engineered to replace endogenous EphB2 in an EphB3 null background. The results show that the proliferative effects of EphB2 require its kinase activity. Further analyses demonstrated that EphB2 relies on Abl kinase activation and consequent upregulation of cyclin D1 levels to promote proliferation (Figure 1). Abl was previously identified as a critical effector of the related EphB4 receptor in breast cancer cells. However, in those cells Abl signals by phosphorylating and inactivating the adaptor protein Crk, thereby decreasing both proliferation and migration (Noren et al., 2006). Eph-Abl functions may therefore vary depending on the cellular context or the receptor involved. It will be interesting to dissect the signaling connection by which Abl regulates cyclin D1, and why the well-characterized Abl substrate Crk does not seem to participate in EphB signaling in intestinal cells. Another interesting issue is

whether EphB-mediated proliferation in adenomas depends only on ephrin-Bs from normal epithelium or also on low levels of coexpressed ephrin-Bs.

The microarray data also revealed that EphB receptors increase transcription of p110α, the most abundant PI3 kinase catalytic subunit isoform expressed in the colon (Figure 1). Treatment of adenoma-like cells and mice with the PI3 kinase inhibitor LY294002 demonstrated a requirement for PI3 kinase activity in EphB-dependent repulsion. Surprisingly, the positioning of the secretory Paneth cells at the bottom of the crypts appears to be regulated by EphB2 through a kinase-independent pathway, as demonstrated by using forms of the receptor mutated in the kinase domain (Genander et al., 2009; Holmberg et al., 2006). Similarly, EphA8 can increase the levels of the p110γ isoform through kinase-independent regulation of protein stability (Gu and Park, 2003). The resulting integrin activation could provide a cell context-dependent mechanism for either promoting or inhibiting cell migration. It will be interesting to further characterize the interplay between EphB2 and PI3 kinase, and determine whether it requires the p85 subunit of PI3 kinase and involves regulation of PI3 kinase catalytic activity (Figure 1). Alternatively, EphB2 may stimulate a more complex migratory program that only requires basal PI3 kinase activity and perhaps involves Rac or E-cadherin (Batlle et al., 2002; Cortina et al., 2007; Miao et al., 2005). An interesting prediction is that the EphB6 receptor, which lacks kinase activity and is also expressed at the bottom of crypts (Kosinski et al., 2007), would possess the tumor suppressing but not the proliferative activity.

The new findings have potential therapeutic implications. They suggest that kinase inhibitors targeting EphB receptors and Abl would have beneficial effects against colorectal cancer because they would inhibit proliferation without affecting tumor confinement by ephrins. Eph kinase inhibitors could be useful to slow the growth of adenomas, where EphB receptors are highly expressed. Abl kinase inhibitors, such as imatinib, could additionally inhibit proliferation in the more malignant carcinomas, where Abl and cyclin D1 are still active even if uncoupled from EphB receptors (Genander et al., 2009). Dasatinib, another Abl inhibitor approved for human use, also potently inhibits Eph receptors as well as Src and could therefore be more effective. A caveat is that different Eph receptors may play different roles in colorectal cancer. For instance, it is controversial whether EphB2/EphB3 and EphB4 have similar or contrasting roles in the intestine (Batlle et al., 2005; Kumar et al., 2009). Promoting EphB kinase activity, although more challenging, could instead be useful for regenerative medicine.

Through a tour-de-force of in vivo studies with a wide collection of mouse models, Genander et al. highlight a new facet of the complex Eph roles in cancer cells: the ability to simultaneously activate distinct pathways with contrasting effects. The novel signaling connections identified in an in vivo physiological context may involve multiple steps and additional aspects that will be important to dissect further. The role of "reverse" signals transduced by the transmembrane ephrin-Bs in intestinal cells also awaits investigation.

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