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Deciphering tumor-suppressor signaling in flies: Genetic link between Scribble/Dlg/Lgl and the Hippo pathways

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Abstract

Loss of apico-basal polarity is one of the crucial factors that drives epithelial tumor progression. *scribble/discs large/lethal giant larvae* (*scrib/dlg/lgl*), a group of apico-basal polarity genes, were initially identified as members of "neoplastic" tumor-suppressors in flies. The components of the Hippo signaling pathway, which is crucial for organ size control and cancer development, were also identified through *Drosophila* genetic screens as members of "hyperplastic" tumor-suppressors. Accumulating evidence in recent studies implies that these two tumor-suppressor signaling pathways are not mutually exclusive but rather cooperatively act to give rise to highly malignant tumors. The interaction of these tumor-suppressor pathways could include deregulations of actin cytoskeleton, cell−cell contact, and apical-domain size of the epithelial cell.

Keywords: Cell polarity; Hippo pathway; *Drosophila*; *scribble*; *dlg*; *lgl*; Tumor-suppressor; Tumor progression

1. Introduction

Most cancers originate from epithelial tissues. Epithelial cells normally exhibit pronounced apico-basal polarity, which is essential for the maintenance of normal cellular functions and epithelial homeostasis. Loss of cell polarity in epithelium is frequently associated with cancer development and progression (Fish and Molitoris, 1994; Bissell and Radisky, 2001). In this sense, genes essential for establishing apico-basal polarity are considered to be tumor-suppressors. Indeed, the first identified tumor-suppressor gene, the *Drosophila lethal giant larvae* (*lgl*) gene, was later demonstrated to be a cell polarity gene (Gateff and Schneiderman, 1967; Bilder et al., 2000). Intriguingly, a single loss-of-function mutation in *lgl* gene is sufficient to develop multilayered and invasive tumors in *Drosophila* imaginal epithelia; it is therefore called "neoplastic" tumor-suppressor gene (Bilder, 2004; Hariharan and Bilder, 2006). The second and the third neoplastic tumor-suppressors, *discs large* (*dlg*) and *scribble* (*scrib*), which act together with *lgl* in a single genetic pathway that controls epithelial cell polarity, have also been isolated from the classical *Drosophila* mutants (Gateff and Schneiderman, 1967; Woods and Bryant, 1989; Bilder and Perrimon, 2000). Further studies identified other neoplastic tumor-suppressors such as *avalanche* (*avl*), *rab5*, *tsg101* and *vps25* (Lu and Bilder, 2005; Moberg et al., 2005; Thompson et al., 2005; Vaccari and Bilder, 2005). Genetic mosaic technique available in *Drosophila* enables to produce a human cancer situation in which clones of oncogenic mutant cells are generated in otherwise wild-type tissue (Xu and Rubin, 1993). Intriguingly, the neoplastic tumor-suppressor mutants do not overproliferate when surrounded by wild-type tissue; instead, these mutant clones are eliminated from epithelium by cell competition (Woods and Bryant, 1991; Agrawal et al., 1995; Brumby and Richardson, 2003). Simultaneous expression of oncogenic Ras (Ras^{V12}) in these mutant clones not only rescues the elimination but also transforms them into metastatic tumors

Abbreviations: AJ, Adherence junction; aPKC, Atypical protein kinase C; Crb, Crumbs; Dlg, Discs large; Ex, Expanded; F-actin, Filamentous actin; Ft, Fat; Hpo, Hippo; JNK, c-Jun N-terminal kinase; Lgl, Lethal giant larvae; Sav, Salvador; Scrib, Scribble; Upd, Unpaired; Wts, Warts; YAP, Yes-associated protein; Yki, Yorkie.

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(Brumby and Richardson, 2003; Pagliarini and Xu, 2003), representing a phenomenon of "multistep carcinogenesis."

Genetic screens in *Drosophila* using the genetic mosaic technique have also identified a series of "hyperplastic" tumor-suppressor genes. These genes include components of the Hippo signaling pathway such as *warts* (*wts*)/*lats*, *salvador* (*sav*)/*shar-pei*, *hippo* (*hpo*) and *mob as tumor suppressor* (*mats*) as well as *Tsc1* and *archipelago* (*ago*) (Justice et al., 1995; Xu et al., 1995; Gao and Pan, 2001; Moberg et al., 2001; Potter et al., 2001; Kango-Singh et al., 2002; Tapon et al., 2002; Harvey et al., 2003; Jia et al., 2003; Pantalacci et al., 2003; Udan et al., 2003; Wu et al., 2003; Lai et al., 2005). Unlike "neoplastic" tumor-suppressors, these mutations are sufficient to trigger overgrowth when surrounded by wild-type tissue. Furthermore, in contrast to highly malignant and undifferentiated "neoplastic" tumors, these "hyperplastic" tumors are normally shaped and maintain the characteristics of an epithelial monolayer, ultimately differentiating into adult tissue. Thus, it seems that different machineries leading to "neoplastic" or "hyperplastic" tumor formation have been installed in fly epithelium.

However, recent works suggest that these two tumor-suppressor signaling pathways, the Scrib/Dlg/Lgl pathway and the Hippo pathway, are genetically linked with each other in tissue growth regulation. In this review, we first describe the molecular machineries of these two tumor-suppressor pathways and then focus on discussing the possible mechanisms whereby Scrib/Dlg/Lgl polarity proteins regulate epithelial tissue growth by modulating the Hippo signaling pathway.

2. Scrib/Dlg/Lgl neoplastic tumor-suppressors as polarity proteins

lgl was initially identified as a gene responsible for mutants that cause tumorous overgrowth in imaginal epithelia (Gateff and Schneiderman, 1967; Gateff, 1978), and was later recognized as a gene essential for establishing apico-basal polarity (Bilder et al., 2000). Tumors developed in *lgl* mutant larvae show multilayered and invasive tumors and are called *Drosophila* "neoplastic" tumors. *dlg* and *scrib* were also identified as "neoplastic" tumor-suppressors, as their mutations lead to the formation of similar tumors with disorganized cell architecture in imaginal epithelia (Bilder et al., 2000; Bilder, 2004; Humbert et al., 2008). *scrib/dlg/lgl* genes all encode cytoplasmic proteins associated with cell membrane. Scrib is a membrane-associated scaffolding protein bearing 16 N-terminal leucine-rich repeats (LRRs) and four PDZ domains (a member of the LAP family protein) (Bilder and Perrimon, 2000). Scrib localizes to the basolateral cortex, which is basal to adherens junction (AJ) at the basolateral region. Dlg is a scaffolding protein of the MAGUK family, which contains three PDZ domains, an SH3 domain, and a GUK domain (Woods and Bryant, 1991). Dlg directly binds to Scrib and co-localizes with Scrib at the basolateral cortex (Hough et al., 1997; Bilder and Perrimon, 2000). Unlike Dlg and Scrib, Lgl, a WD40 repeat protein, is not a scaffold protein (Jacob et al., 1987). Lgl essentially localizes at the plasma membrane and its localization is affected by its phosphorylation. Lgl can be phosphorylated by atypical protein kinase C (aPKC), and the phosphorylated Lgl is unable to localize at the cortex (Betschinger et al., 2005; Wirtz-Peitz and Knoblich, 2006). Lgl loses its plasma membrane localization in *scrib* or *dlg* mutant embryos, suggesting a critical role of Scrib and Dlg in recruiting Lgl to the plasma membrane. Scrib, Dlg and Lgl cooperatively contribute to establishing epithelial cell polarity (Bilder and Perrimon, 2000). Genetic studies in *Drosophila* have identified other polarity protein complexes including the aPKC-Par-6-Bazooka (Baz, a Par-3 homolog) complex and the Crumbs (Crb)-Patj-Stardust (Sdt, a Pals1 homolog) complex (Knust and Bossinger, 2002). Genetic studies have elucidated the antagonistic regulation among the Scrib/Dlg/Lgl module, the aPKC complex, and the Crb complex in embryonic epithelium (Bilder et al., 2003; Tanentzapf and Tepass, 2003). The aPKC complex represses the activity of Scrib/Dlg/Lgl by excluding Lgl from the apical cortex, while the Scrib/Dlg/Lgl module inhibits aPKC complex at the basolateral region, which contributes to distinguishing the basal domain from the apical domain. The aPKC complex activates the Crb complex by recruiting Crb to the apical region, and in turn, the Crb complex inhibits Scrib/Dlg/Lgl. Recently, a new complex, the Yurt (Yrt)/Coracle (Cora)/Neurexin IV (Nrx-IV)/Na^+, K^+-ATPase complex, which is a second group of basolateral proteins, has been added to the polarity complexes (Laprise et al., 2009). Yrt/Cora/Nrx-IV/Na^+, K^+-ATPase complex stabilizes the basolateral membrane and negatively regulates the activity of the Crb complex (Laprise et al., 2009). All four polarity complexes contribute to establishing apico-basal axis in epithelial cells by mutually modulating each complex's activity (Nelson, 2003; Bilder, 2004; Laprise and Tepass, 2011) (Fig. 1).

3. Clone phenotypes of Scrib, Dlg and Lgl

Cancers develop from a small number of oncogenic cells as genetic mosaic. Therefore, at early stages of epithelial cancer development, clones of oncogenic cells are surrounded by normal cells. The interaction between oncogenic and normal cells is crucial for tumor development. For instance, clones of cells mutant for any one of the Hippo pathway components overgrow not only by stimulating cell proliferation but also by eliminating surrounding wild-type cells through cell competition (Tyler et al., 2007) (Fig. 2A). Intriguingly, although imaginal tissues entirely mutant for *scrib*, *dlg* or *lgl* overgrow and develop into tumors (Fig. 2B), these neoplastic tumor-suppressor mutants do not grow when surrounded by wild-type tissue; instead, these mutant clones are eliminated by cell competition (Agrawal et al., 1995; Brumby and Richardson, 2003; Igaki et al., 2009). This elimination of neoplastic tumor-suppressor mutants is driven by Eiger (a TNF homolog)−c-jun N-terminal kinase (JNK) signaling. In *scrib* mutant clones surrounded by wild-type tissue, Eiger−JNK apoptotic signaling is activated to promote elimination of these cells, while in the surrounding wild-type cells, the Eiger−JNK-mediated engulfment pathway is activated to