Cell Research (2010) 20:242-245.

www.nature.com/cr

LETTER TO THE EDITOR

Activation of JNK signaling links *lgl* mutations to disruption of the cell polarity and epithelial organization in *Drosophila* imaginal discs

Cell Research (2010) **20**:242-245. doi: 10.1038/cr.2010.2; published online 12 January 2010

Dear Editor,

Identification of *Drosophila melanogaster* as a model organism for cancer research has facilitated the exploration of human tumor malignancy. In *Drosophila*, loss-of-function mutations in the neoplastic tumor suppressor genes (nTSGs) lethal(2)giant larvae (*lgl*), discs large (*dlg*) or scribble (*scrib*) cause a malignant tumor-like phenotype characteristic of disrupted cell polarity and overgrowth in epithelial tissues such as imaginal discs [1]. Genetic studies have shown that the three nTSGs regulate the epithelial polarity and cell proliferation in *Drosophila* development through a common genetic pathway [2]. However, the underlying regulatory mechanisms are largely unknown.

Early on it was postulated that overgrowth is the secondary consequence of defective apicobasal polarity in nTSG-deficient epithelial cells [1]. The coupling of the polarity to proliferation control may be mediated through signaling pathways. Indeed, two laboratories recently reported that the c-Jun N-terminal kinase (JNK) signaling activity is essential in both tumor growth and invasion induced by $nTSG^{-/-}$ clones expressing Ras^{V12}, the oncogenic form of Ras [3, 4]. In Ras^{V12}, $nTSG^{-/-}$ clonal tumors, however, the genetic evidence for the causal relationship between loss of cell polarity and activation of JNK signaling appears to be less substantial. To better understand how JNK signaling contributes to the tumorigenesis induced by nTSG mutations, we used *lgl* homozygous mutant flies as an alternative to dissect the function of the JNK signaling pathway in each of the tumor phenotypes.

First, we tested if the germ-line mutations in *lgl* can cause JNK activation during *Drosophila* development. For this purpose, we generated fly lines trans-heterozygous for $lgl^4/Df(2L)net62$ or lgl^4/lgl^1 (hereafter referred to as *lgl* flies) in the presence of an enhancer trap line, puc^{E69}-*lacZ*. As expression of puc^{E69}-*lacZ* is considered as an indicator of JNK signaling activation in *Drosophila* [5], we thereby assessed the activity of JNK signaling pathway in *lgl* imaginal discs by monitoring β-galactosidase expression. As shown in Figure 1A, localized expression of *puckered* (*puc*) in stalk cells and some peripodial membrane cells was observed in the wild-type wing disc at the late third instar larvae (Figure 1A-i), whereas the ectopic activation of JNK pathway was evident in nearly all the cells in *lgl* imaginal discs at the same stage, including wing, eye-antennal, leg and haltere discs (Figure 1A-ii and iv; data not shown). Clearly, loss of *lgl* alone can activate JNK signaling in the developing discs, suggesting a role of JNK activity in *lgl* mutation-induced tumorigenesis similar to that in the development of Ras^{V12}, $nTSG^{-/-}$ clonal tumors.

To analyze the potential function of JNK signaling in *lgl* tumor development, we next inhibited JNK activation in *lgl* flies by expressing a dominant-negative form of *Drosophila* JNK (Basket, Bsk), Bsk^{DN}, using the UAS/GAL4 system [6]. The flies homozygous for *lgl* show a typical growth pattern throughout larval development. Instead of pupating on day 5 after egg laying (AEL), *lgl* flies continued to grow for up to 10 additional days and finally died as giant larvae (Supplementary information, Table S1). During this extended larval period, *lgl* imaginal discs become overgrown (Supplementary information, Figure S1). In our experiments, *32B-Gal4*-driven expression of *UAS-*bsk^{DN} in *lgl* flies resulted in phenotypic reversion of the tumor-like overgrowth as characterized by "rescued" size of the mutant discs, absence of the giant larvae and recurrence of pupariation (Supplementary information, Table S1 and Figure S1). Likewise, inhibiting JNK can largely suppress the invasion-like behavior of *lgl* tumorous discs, as indicated by the absence of a fusion between the adjacent mutant discs expressing Bsk^{DN} under the control of *32B-Gal4* (Supplementary information, Figure S1). Taken together, the results of our study revealed that JNK activation is required for both tumor

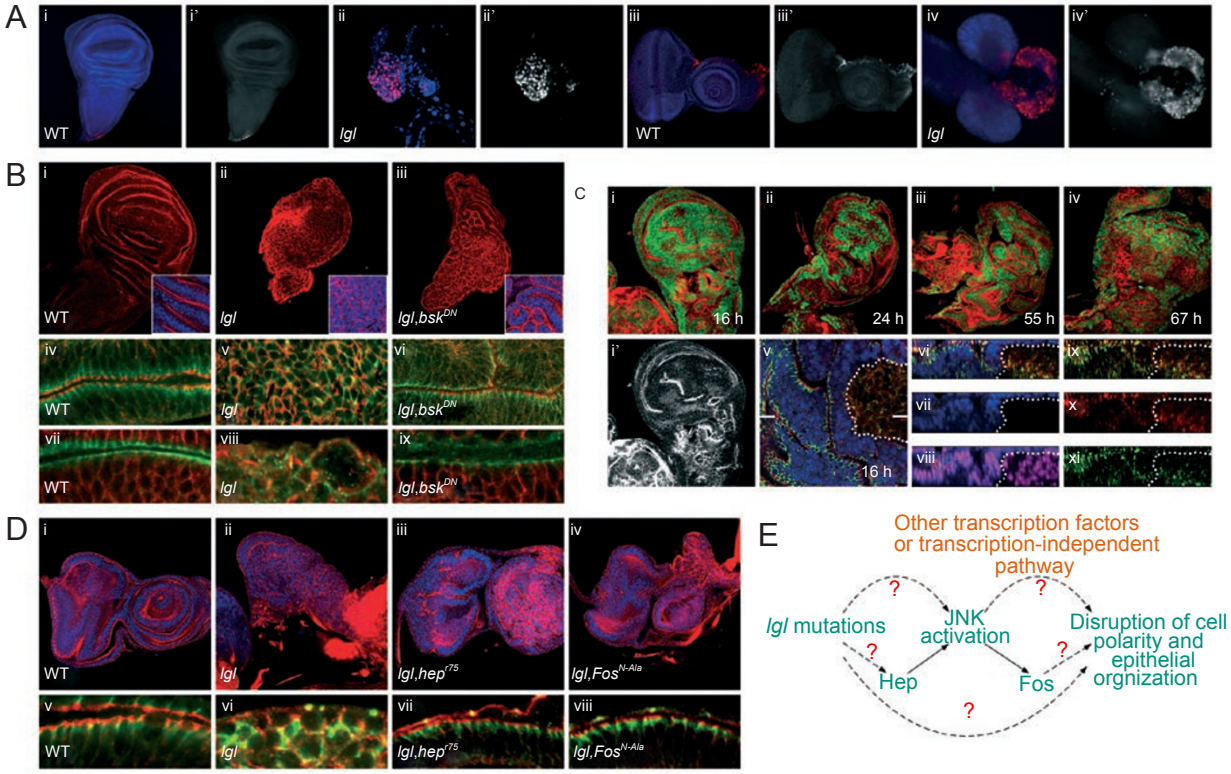

Figure 1 Inhibiting JNK pathway partially rescues the defects in tissue architecture and epithelial polarity caused by *lgl* mutation. **(A)** JNK signaling was ectopically activated in *lgl*^{4}*/Df*(*2L*)*net62* wing (ii and ii') and eye-antennal discs (iv and iv'), as indicated by *puc*^{E69}*-lacZ* expression (red in i-iv and white in I'-iv'). Note that magnification is ×140 for i and ×200 for ii-iv. **(B)** 32B-Gal4-driven expression of *bsk*^{DN} in *lgl*^{4}*/Df*(*2L*)*net62* flies partially rescued the epithelial organization and cell polarity of the wing discs (compare iii, vi and ix with ii, v and viii, respectively), as revealed in the presence of monolayered cell sheets with columnar cell shape, polarized accumulation of F-actin (red in i-iii) and properly localized cell polarity markers (Arm, red in iv-vi; Dlg, green in iv-vi; aPKC, green in vii-ix; Fas3, red in vii-ix). **(C)** JNK activation locally disrupts the epithelial polarity of the *lgl* discs in a time-dependent manner during development. *lgl*^{4}*/lgl*^{1} wing discs with FLP-out clones (GFP-positive, green in i-iv and blue in v-viii) expressing *bsk*^{K53R} at various developmental stages (the time when clone was induced is marked on the photo), stained for F-actin (red in i-iv and white in i') or Arm (red in v-xi), Dlg (green in v-xi), showed a restoration in the epithelial organization and cell polarity to a certain extent which is closely correlated with the timing and pattern of *bsk*^{K53R} expression. Note that vi-xi is the XZ optical cross section at the level of the white lateral marks in v. GFP-negative cells are outlined with dots in v-xi. **(D)** The presence of *hep*^{r75}, or 32B-Gal4-driven expression of *Fos*$^{N-Ala}$ (a dominant-negative form of dFos) partially restored the epithelial organization and cell polarity of the *lgl*^{4}*/Df*(*2L*)*net62* eye-antennal discs, as revealed by F-actin (red in i-iv) or Arm (red in v-viii) and Dlg (green in v-viii) staining. Note that magnification is ×200 for i and ×280 for ii-iv. In all panels above, nuclei are stained with DAPI (blue in **A**, **B**, **D** and purple in **C**). **(E)** A hypothetical model for the JNK-dependent mechanisms underlying disruption of the cell polarity and epithelial organization induced by *lgl* mutations.

growth and invasion of *lgl* imaginal discs, which is reminiscent of the observations in Ras^{V12}, nTSG$^{-/-}$ clonal tumors.

The *Drosophila* imaginal disc is comprised of a monolayer of elongated and polarized epithelial cells, forming a flat and folded disc (Figure 1B-i). Loss of *lgl* usually disrupts both epithelial polarity and tissue organization in the disc, showing a solid amorphous mass piled up with rounded, multilayered cells with defective apicobasal polarity (Figure 1B-ii). Thus, *lgl* discs can provide a good system for studying the causal link between polarity disruption and the JNK signaling pathway in tumor development. As described above, we found that the ectopic JNK activity is essential for overgrowth and invasion of *lgl* tumorous discs. Further examination of the rescued discs, however, revealed a partial restoration of the epithelial disorganization observed in *lgl*-deficient discs as well. JNK inhibition, on the whole, can bring back the overall shape of *lgl* discs close to the wild type (Figure 1B-i-iii). Remarkably, in *lgl* discs expressing Bsk^{DN} under the control of *32B-Gal4*, phalloidin staining showed the presence of a partially folded epithelial

sheet in which the F-actin was apically accumulated in the monolayered cells with columnar morphology (inset in Figure 1B-iii). The F-actin localization pattern in the rescued discs suggests a role of JNK activation in disruption of epithelial polarity triggered by loss of *lgl*. This prompted us to further investigate how the apicobasal polarity in *lgl* discs expressing Bsk^{DN} is restored by examining the subcellular localization of selected epithelial polarity proteins in the disc cells. Consistently, we observed a proper distribution of a series of the polarity proteins such as Armadillo (Arm, zonula adherens protein), Dlg (septate junction protein), aPKC (apical protein) and Fasciclin3 (Fas3, basolateral protein) along the apicobasal axis in epithelial cells of the rescued discs (Figure 1B-iv-ix). The restoration in tissue architecture and epithelial polarity was also obtained by expression of another dominant-negative allele of JNK (bsk^{K53R}), bsk^{RNAi} or the JNK-specific phosphatase Puc in *lgl* discs under the control of *32B*, *69B* or *eyeless Gal4*, respectively (data not shown), albeit to a lesser extent. Collectively, these data provide evidence that JNK signaling activated by loss of *lgl* mediates disruption of the epithelial polarity and tissue organization in the mutant discs in addition to promoting tumor growth.

We further characterized the role of JNK activation in disrupting the epithelial polarity in *lgl* imaginal discs by expressing Bsk^{DN} in a temporally and spatially regulated manner, based on the FLP-out system [7]. When ectopic expression of Bsk^{DN} was induced as early as 16 h AEL, we detected a clear restoration in both epithelial polarity and organization, as shown by the presence of polarized epithelial monolayers in the discs (Figure 1C-i). Moreover, the expression pattern of Bsk^{DN} and the restored area match well in those rescued discs (Figure 1C-i, v-xi). To explore whether activated JNK signaling in *lgl* flies functions in a time-dependent manner during development, we performed a kinetic assay through suppressing JNK activities in nearly the whole tissue of *lgl* wing disc at different developmental stages. Interestingly, we found that the later the Bsk^{DN} expression was induced, the lesser the epithelial organization in *lgl* wing discs was restored (Figure 1C-i-iv), suggesting a temporally regulated action of JNK signaling in the pathological process. Altogether, these findings suggest that loss of *lgl* induces activation of JNK signaling at early stage of the development to mediate locally the disruption of epithelial polarity and tissue organization, and that the duration of JNK activation is closely correlated with phenotypic severity.

It is known that JNK signaling mediates a number of cellular and developmental processes through activating an evolutionarily conserved pathway. To address whether the canonical JNK pathway is responsible for the disruption of cell polarity and epithelial organization observed in *lgl*-deficient discs, we first targeted Hemipterous (Hep), the *Drosophila* JNK kinase for inactivation by introducing hep^{r75}, a strong hypomorphic allele into *lgl* flies [8]. Examination of the imaginal discs from hep^{r75}/Y; $lgl^{4}/DF(2L)net62$ larvae revealed a weaker restoration in apicobasal polarity of disc cells and disc morphology, compared with that in *lgl* discs expressing Bsk^{DN} (Figure 1D-iii and vii; data not shown). We also investigated whether JNK-mediated effects in the polarity disruption and epithelial disorganization require AP-1 transcription factors. We found that inactivation of dFos by expressing either dominant-negative forms of the gene or an RNAi transgene can phenocopy the suppressive effects conferred by JNK inhibition (Figure 1D-iv and viii; data not shown). On the basis of these observations, we argue that an activated Hep-JNK-AP-1 pathway is involved in mediating *lgl* mutation-induced tumorigenesis regarding the loss of epithelial polarity and disruption of tissue organization (Figure 1E).

This study showed that JNK signaling is an essential mediator of many tumorous phenotypes observed in *lgl*-deficient imaginal discs. In particular, we present evidence that activation of the JNK signaling pathway is causally linked to the disruption of apicobasal polarity and epithelial organization in the mutant tissues. It has been established that JNK could function as an either positive or a negative regulator in cancer development, depending on the tumor cell type, specific genetic setting or tumorigenic stage involved [9]. Although JNK regulation of a number of biological processes such as morphogenesis, cell proliferation, migration, adhesion and apoptosis is implicated in human tumor malignancy, the exact role of JNK signaling in tumorigenesis is not yet fully understood. Given that defects in cell polarity are frequently seen in human malignant tumors [10], our finding in the *Drosophila* model that loss of *lgl* activates the JNK pathway to mediate the disruption of cell polarity and epithelial organization may suggest an evolutionarily conserved mechanism, thereby providing new insights into the involvement of JNK signaling in human cancer development.

Acknowledgments

We thank Michel Sémériva (IBDML, France), Fumio Matsuzaki (RIKEN CDB, Japan), Michael J Galko (UT MD Anderson Cancer Center, USA), Enrique Martín-Blanco (Instituto de Biologia Molecular de Barcelona, Spain), Dirk Bohmann (University of Rochester, USA), Kyung-Ok Cho (Baylor College of Medicine, USA), the Bloomington *Drosophila* Stock Center, NIG-FLY (Japan), and the Developmental Studies Hybridoma Bank

for providing fly strains and antibodies. We also thank Wenjuan Xiang and Wentian Gu in ML's Lab for the technical assistance. This work was supported by National Natural Science Foundation of China (30470890), National Basic Research Program of China (2007CB914504, 2007CB947301), the Shanghai Pujiang Program (05PJ14075) and Shanghai Leading Academic Discipline Project (B205).

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References

1 Bilder D. Epithelial polarity and proliferation control: links from the *Drosophila* neoplastic tumor suppressors. *Genes Dev* 2004; **18**:1909-1925.
2 Bilder D, Li M, Perrimon N. Cooperative regulation of cell polarity and growth by *Drosophila* tumor suppressors. *Science* 2000; **289**:113-116.
3 Igaki T, Pagliarini RA, Xu T. Loss of cell polarity drives tumor growth and invasion through JNK activation in *Drosophila*. *Curr Biol* 2006; **16**:1139-1146.
4 Uhlirova M, Bohmann D. JNK- and Fos-regulated Mmp1 expression cooperates with Ras to induce invasive tumors in *Drosophila*. *EMBO J* 2006; **25**:5294-5304.
5 Martin-Blanco E, Gampel A, Ring J, *et al*. *puckered* encodes a phosphatase that mediates a feedback loop regulating JNK activity during dorsal closure in *Drosophila*. *Genes Dev* 1998; **12**:557-570.
6 Brand AH, Perrimon N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 1993; **118**:401-415.
7 Struhl G, Basler K. Organizing activity of wingless protein in *Drosophila*. *Cell* 1993; **72**:527-540.
8 Glise B, Bourbon H, Noselli S. *hemipterous* encodes a novel *Drosophila* MAP kinase kinase, required for epithelial cell sheet movement. *Cell* 1995; **83**:451-461.
9 Wagner EF, Nebreda AR. Signal integration by JNK and p38 MAPK pathways in cancer development. *Nat Rev Cancer* 2009; **9**:537-549.
10 Wodarz A, Nathke I. Cell polarity in development and cancer. *Nat Cell Biol* 2007; **9**:1016-1024.

(**Supplementary information** is linked to the online version of the paper on the *Cell Research* website.)