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Report

Loss of Cell Polarity Drives Tumor Growth and Invasion through JNK Activation in *Drosophila*

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Summary

Apparent defects in cell polarity are often seen in human cancer [1, 2]. However, the underlying mechanisms of how cell polarity disruption contributes to tumor progression are unknown. Here, using a *Drosophila* genetic model for Ras-induced tumor progression, we show a molecular link between loss of cell polarity and tumor malignancy. Mutation of different apicobasal polarity genes activates c-Jun N-terminal kinase (JNK) signaling and downregulates the E-cadherin/β-catenin adhesion complex, both of which are necessary and sufficient to cause oncogenic Ras^{V12}-induced benign tumors in the developing eye to exhibit metastatic behavior. Furthermore, activated JNK and Ras signaling cooperate in promoting tumor growth cell autonomously, as JNK signaling switches its proapoptotic role to a progrowth effect in the presence of oncogenic Ras. Our finding that such context-dependent alterations promote both tumor growth and metastatic behavior suggests that metastasis-promoting mutations may be selected for based primarily on their growth-promoting capabilities. Similar oncogenic cooperation mediated through these evolutionarily conserved signaling pathways could contribute to human cancer progression.

Results

Most human cancers originate from epithelial tissues. These epithelial tumors, except for those derived from squamous epithelial cells, normally exhibit pronounced apicobasal polarity. However, these tumors commonly show defects in cell polarity as they progress toward malignancy [1, 2]. Although the integrity of cell polarity is essential for normal development [3], how cell polarity disruption contributes to the signaling mechanisms essential for tumor progression and metastasis is unknown. To address this, we used a recently established *Drosophila* model of Ras-induced tumor progression triggered by loss of cell polarity. This fly tumor model exhibits many aspects of metastatic behaviors observed in human malignant cancers, such as basement membrane degradation, loss of E-cadherin expression, migration, invasion, and metastatic spread to other organ sites [4]. In the developing eye tissues of these animals, loss of apicobasal polarity is induced by disruption of evolutionarily conserved cell polarity genes such as *scribble* (*scrib*), *lethal giant larvae* (*lgl*), or *discs large* (*dlg*), three polarity genes that function together in a common genetic pathway, as well as other cell polarity genes such as *bazooka*, *stardust*, or *cdc42* [3, 5]. Oncogenic Ras (Ras^{V12}), a common alteration in human cancers [4, 6], causes noninvasive benign overgrowths in these eye tissues [4]. Loss of any one of the cell polarity genes somehow strongly cooperates with the effect of Ras^{V12} to promote excess tumor growth and metastatic behavior [4, 7]. However, on their own, clones of *scrib* mutant cells are eliminated during development in a JNK-dependent manner; expression of Ras^{V12} in these mutant cells prevents this cell death [7].

To better quantify the metastatic behavior of tumors in different mutant animals, we focused our analysis on invasion of the ventral nerve cord (VNC), a process in which tumor cells leave the eye-antennal discs and optic lobes (the areas where they were born) and migrate to and invade a different organ, the VNC. We further confirmed that the genotypes associated with the invasion of the VNC in this study also resulted in the presence of secondary tumor foci at distant locations, although the number and size of these foci were highly variable, as previously reported [4].

Loss of Cell Polarity Activates JNK Signaling that Is Essential for Tumor Invasion

In analyzing the global expression profiles of noninvasive and invasive tumors induced in *Drosophila* developing eye discs, we observed that expression of the JNK phosphatase *puckered* (*puc*) was strongly upregulated in the invasive tumors (our unpublished data). Upregulation of *puc* represents activation of the JNK pathway in *Drosophila* [8–10]. We therefore utilized an enhancer-trap allele, *puc-LacZ* [11], to monitor the activation of JNK signaling in invasive tumor cells. Strong ectopic JNK activation was present in invasive tumors (Figures 1D–1F), while only a slight expression of *puc* was seen in restricted regions of Ras^{V12}-induced noninvasive overgrowth (Figures 1A–1C). Intriguingly, more intense JNK activation was seen in tumor cells located in the marginal region of the eye-antennal disc and tumor cells invading the VNC (Figures 1D–1I, arrowheads, and also see Figure S1 in the Supplemental Data available with this article online). Analysis of clones of cells with a cell polarity mutation alone revealed that JNK signaling was activated by mutation of cell polarity genes (Figures 1J–1O). Notably, JNK signaling was not activated in a strictly cell-autonomous fashion (Figures 1M–1O, arrowheads). JNK activation in these cells was further confirmed by anti-phospho-JNK antibody staining that detects activated JNK (see Figure S2).

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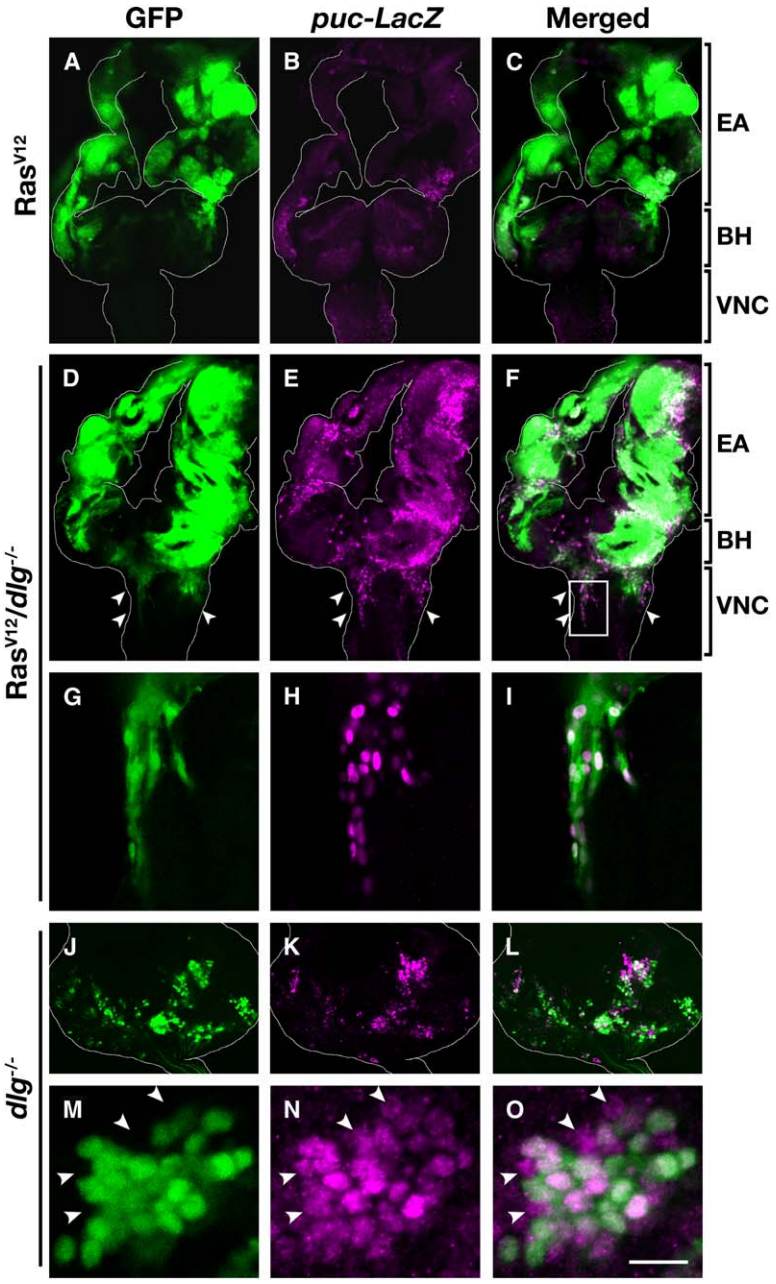

Figure 1. Invading Cells Are Activating JNK, which Is Triggered by Loss of Cell Polarity

JNK activation was determined by anti-β-galactosidase staining (magenta) in the genetic background of *puc-LacZ* in GFP-labeled Ras^{V12} benign overgrowth (A–C), Ras^{V12}/$dlg^{-/-}$ invasive tumors (D–I), or cell polarity-deficient $dlg^{-/-}$ clones (J–O) (green). Confocal images of the day 6 cephalic complexes, which include the eye-antennal discs (EA), brain hemispheres (BH), the VNC (see also Figure 2) (A–I), and the day 4 eye discs (J–O) are shown. JNK is strongly activated in invading cells in the BH or the VNC ([D]–[I] and arrowheads. (G)–(I) are high-magnification images shown by a square in (F). High magnification of $dlg^{-/-}$ clones shows some non-cell-autonomous upregulation of *puc* ([M]–[O], arrowheads; scale bar, 10 μm). n > 10 for each genotype. See Supplemental Data for genotypes.

To examine the contribution of JNK activation to metastatic behavior, we blocked the JNK pathway by overexpressing a dominant-negative form of *Drosophila* JNK (Bsk^{DN}). As previously reported [4], clones of cells mutant for *scrib*, *lgl*, or *dlg* do not proliferate as well as wild-type clones (Figures 2A–2D), while combination of these mutations with Ras^{V12} expression resulted in massive and metastatic tumors (Figures 2G, 2G′, 2I, 2I′, 2K, and 2K′). Strikingly, inhibition of JNK activation by Bsk^{DN} completely blocked the invasion of the VNC (Figures 2G′–2L′), as well as secondary tumor foci formation (see Figure S3). *Drosophila* has two homologs of TRAF proteins (DTRAF1 and DTRAF2), which mediate signals from cell surface receptors to the JNK kinase cascade in mammalian systems [12]. We found that RNAi-mediated inactivation of DTRAF2, but not DTRAF1, in the tumors strongly suppressed their metastatic behavior (Figures 2M′ and 2N′). Inactivation of dTAK1, a *Drosophila* JNK kinase kinase (JNKKK), or Hep, a JNKK, also suppressed metastatic behavior (Figures 2O′ and 2P′). *Drosophila* has two known cell surface receptors that act as triggers for the JNK pathway, Wengen (TNF receptor) and PVR (PDGF/VEGF receptor) [13, 14]. Intriguingly, we found that RNAi-mediated inactivation of Wengen partially suppressed tumor invasion (Figure 2Q′). Inactivation of PVR, on the other hand, did not show any suppressive effect on metastatic behavior (Figure 2R′). We also found that the metastatic behavior of Ras^{V12}-expressing tumors that were also mutated for one of three other cell polarity genes, *bazooka*, *stardust*, or *cdc42*, was also blocked by Bsk^{DN} (data not shown). These data indicate that loss of cell polarity contributes