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Oncogenic Ras Diverts a Host TNF Tumor Suppressor Activity into Tumor Promoter

Julia B. Cordero,[1] Juan P. Macagno,[1] Rhoda K. Stefanatos,[1] Karen E. Strathdee,[1] Ross L. Cagan,[2] and Marcos Vidal[1,*]
[1]Beatson Institute for Cancer Research, Cancer Research UK. Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD, UK
[2]Department of Developmental and Regenerative Biology, Annenberg 25-40, Mount Sinai Medical School, One Gustave L. Levy Place, Box 1020, NY 10029, USA
*Correspondence: m.vidal@beatson.gla.ac.uk
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SUMMARY

The roles of inflammatory cytokines and the immune response in cancer remain paradoxical. In the case of tumor necrosis factor (TNF), there is undisputed evidence indicating both protumor and antitumor activities. Recent work in *Drosophila* indicated that a TNF-dependent mechanism eliminates cells deficient for the polarity tumor suppressors *dlg* or *scrib*. In this study, however, we show that in tumors deficient for *scrib* that also expressed the Ras oncoprotein, the TNF signal was diverted into a protumor signal that enhanced tumor growth through larval arrest and stimulated invasive migration. In this case, TNF promoted malignancy and was detrimental to host survival. TNF was expressed at high levels by tumor-associated hemocytes recruited from the circulation. The expression of TNF by hemocytes was both necessary and sufficient to trigger TNF signaling in tumor cells. Our evidence suggests that tumors can evolve into malignancy through oncogenic Ras activation and the hijacking of TNF signaling.

INTRODUCTION

The roles of mammalian tumor necrosis factor (TNF) as both protumor and antitumor are well documented. Initially, treatment with TNF induced necrotic death of subcutaneous murine tumors (Balkwill et al., 1986). On the contrary, later findings showed that TNF deficient mice are more resistant than wild-type to developing tumors (Moore et al., 1999). The molecular mechanisms determining such opposing roles of TNF remain largely unclear. One source of uncertainty is the undefined genetic composition and developmental history of the tumor models used.

The fruit fly *Drosophila melanogaster* is an excellent genetically tractable model system to address the complex cell interactions and genetic cooperation that lead to tumor formation and progression (Vidal and Cagan, 2006). Indeed, the discovery of *lethal giant larvae* (*lgl*) in *Drosophila* (Bridges and Brehme, 1944) provided founding evidence for the existence of tumor suppressor genes.

A significant body of work in *Drosophila* has uncovered the complex mechanisms underlying the role of tumor suppressors and oncogenes in tumorigenesis. Animals fully mutant for *lgl* and other polarity tumor suppressor genes from the "*scribble* group" such as *scribble* (*scrib*) and *disc large* (*dlg*) develop tumors within islands of polarized epithelia known as imaginal discs (Bilder and Perrimon, 2000; Humbert et al., 2008; Perrimon, 1988). On the contrary, discrete clonal patches of genotypically $scrib^{-/-}$ cells are eliminated by Jun N-terminal kinase (JNK)-dependent cell death (Brumby and Richardson, 2003). Nevertheless, overexpression of oncogenic Ras (Ras^{V12}) in discrete $scrib^{-/-}$ clones (Ras^{V12}; $scrib^{-/-}$) leads to invasive tumors (Pagliarini and Xu, 2003) in part due to a blockade of JNK-dependent death (Brumby and Richardson, 2005).

Although the JNK-dependent death of *dlg*- or *scrib*-deficient cells provides a mechanism to eliminate tumors, JNK also constitutes a pleiotropic signaling node with multiple roles in development, homeostasis, and the stress response. In the context of Ras^{V12}; $scrib^{-/-}$ tumors *Drosophila* JNK activation results in invasive cell migration (Igaki et al., 2006; Uhlirova and Bohmann, 2006) through the production of the matrix metalloprotease dMMP1 (Page-McCaw et al., 2003; Uhlirova and Bohmann, 2006).

Among the wide palette of cellular events leading to JNK activation is *dTNF/eiger* (*egr*). Egr is the sole *Drosophila* member of the TNF superfamily and its misexpression in imaginal disc cells results in JNK-dependent apoptosis (Igaki et al., 2002; Moreno et al., 2002). Recent work suggests that the immune system also plays a critical role in *Drosophila* tumor models: (1) hemocytes have been shown to associate to Ras^{V12}; $scrib^{-/-}$ tumors and negatively impact tumor growth in $scrib^{-/-}$ animals (Pastor-Pareja et al., 2008); and (2) JNK-dependent cell death in *scrib* or *dlg* clonal patches of cells requires *egr* (Igaki et al., 2009). The later suggests that the role of TNF as a "tumor death factor" is ancient and conserved from *Drosophila* to mammals.

Using a genetically defined tumor model we show that the "tumor promoting" role of mammalian TNF (Moore et al., 1999) is also conserved in *Drosophila*. We found that TNF acts as a tumor promoter in the context of Ras^{V12}; $scrib^{-/-}$ tumors. Furthermore, TNF was expressed by tumor-associated hemocytes and such expression was both necessary and sufficient for pathway activation in tumor cells. Our results provide what we believe to be novel mechanistic insights explaining the contrasting roles of the immune system in tumorigenesis and suggest a strong dependency on the genetic composition of the tumor.

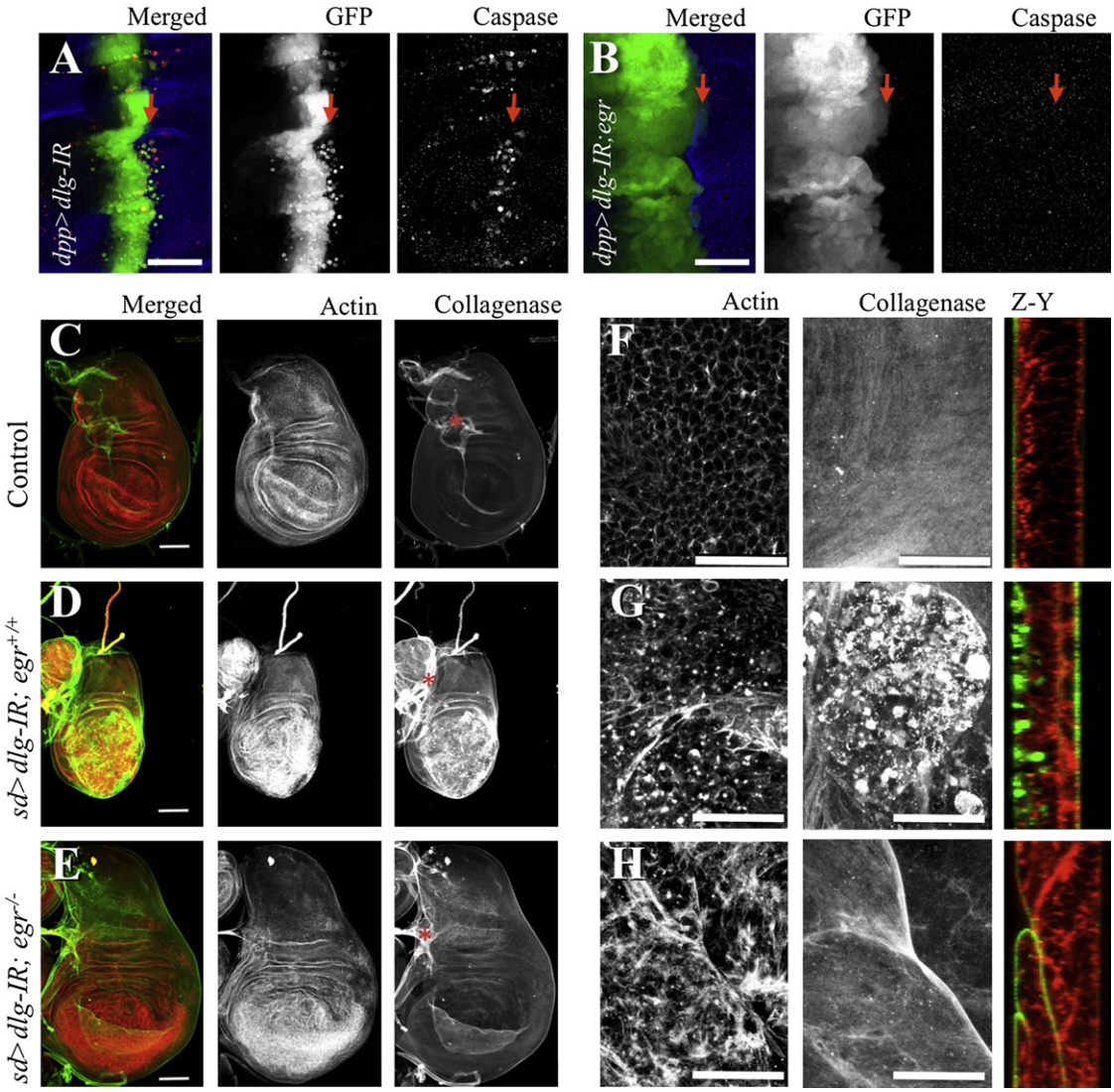

Figure 1. Dlg-Deficient Cell Delamination and ECM Remodeling Are *egr*-Dependent

Confocal images from larval wing discs. Full genotypes for all figures are described in Supplemental Experimental Procedures; relevant genotypes are indicated. In (A) and (B), arrows indicate anterior/posterior (A/P) boundary; the *dpp* expression domain is an anterior stripe at the boundary. Left panels are color overlays from GFP (green), cleaved caspase-3 (red) fluorescent signals; the remaining columns show individual stains in gray as labeled. (C–H) *sd-gal4* was used to drive expression of *dlg-IR* in the wing pouch region from the discs with the indicated *egr* background for each row of panels. Discs were stained for actin filaments (red and center column in C–E, left column in F–H) and collagenase/gelatinase activity in situ to visualize MMP activity (green and right column in C–E, center column in F–H). The right column in (F–H) displays optical cross sections along the Z and Y planes. Asterisks label overlaying trachea branches. Scale bars = (A) and (B), 150 μm, (C)–(E), 100 μm, and (F)–(H), 25 μm. See also Figures S1, S2, and S3.

RESULTS

TNF Is Required for *dlg* Cell Death and Epithelial Delamination

To further analyze the role of *egr* in cells deficient for tumor suppressors of the *scribble* group, we initially combined an *egr* loss of function mutant with an RNA-interference transgene targeting *dlg* (*dlg-IR*). This allowed control of gene expression by using the *gal4/UAS* system (Brand and Perrimon, 1993). *dlg-IR* resulted in decreased levels of the Dlg protein (see Figures S1 and S2 available online), phenocopied *dlg* mutants when ubiquitously expressed (Figure S4), and was rescued by coexpression of Dlg (Figure S2C). Expression of *dlg-IR* under the *decapentaplegic* (*dpp*) or *scalloped* (*sd*) promoters (*dpp* > *dlg-IR* or *sd* > *dlg-IR*) resulted in apoptosis (as assessed by caspase cleavage) (Figures 1A and S2B) and in an invasion-like phenotype in which cells delaminated and migrated away from the site of origin within the wing disc epithelium (Figures 1A and S1A). Despite these phenotypes most *dpp* > *dlg-IR* animals (91%, n = 81) (Figure S1G) survived to adulthood, displaying scars along the anterior/posterior boundary of the wing blade (Figure S1F). In contrast, cell death in *dlg-IR* wing discs was completely blocked in $egr^{-/-}$ animals (Figures 1B and S2C). Instead, the *dpp* domain was enlarged and 100% of $egr^{-/-}$;