Published in final edited form as:
Oncogene. 2011 July 21; 30(29): 3248–3260. doi:10.1038/onc.2011.46.

Sds22/PP1 Links Epithelial Integrity and Tumor Suppression via Regulation of Myosin II and JNK Signaling

Yuwei Jiang[1], **Kenneth L. Scott**[1], **Su-Jin Kwak**[3], **Rui Chen**[1,2,6], and **Graeme Mardon**[1,2,3,4,5,6,*]

[1] Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA

[2] Program in Developmental Biology, Baylor College of Medicine, Houston, TX 77030, USA

[3] Department of Pathology, Baylor College of Medicine, Houston, TX 77030, USA

[4] Department of Neuroscience, Baylor College of Medicine, Houston, TX 77030, USA

[5] Department of Ophthalmology, Baylor College of Medicine, Houston, TX 77030, USA

[6] Human genome Sequencing Center, Baylor College of Medicine, Houston, TX 77030, USA

Abstract

Loss of epithelial integrity often correlates with the progression of malignant tumors. Sds22, a regulatory subunit of Protein Phosphatase 1 (PP1), has recently been linked to regulation of epithelial polarity in *Drosophila*. However, its role in tumorigenesis remains obscure. Here, using *Drosophila* imaginal tissue as an in vivo model system, we show that *sds22* is a new potential tumor suppressor gene in *Drosophila*. Without *sds22*, cells lose epithelial architecture, and become invasive and tumorigenic when combined with Ras overexpression; conversely, *sds22* overexpression can largely suppress tumorigenic growth of $Ras^{V12}scrib^{-/-}$ mutant cells. Mechanistically, we show that *sds22* prevents cell invasion and metastasis by inhibiting myosin II and JNK activity downstream of PP1. Loss of this inhibition causes cells to lose epithelial organization and promotes cell invasion. Finally, human *Sds22* is focally deleted and down-regulated in multiple carcinomas, and this downregulation correlates with tumor progression, suggesting that *sds22* inactivation may contribute to tumorigenesis and metastatic potential in human cancers via a similar mechanism.

Keywords

Tumor suppressor; Epithelial integrity; Cell invasion; *sds22*; Protein Phosphatase 1

Introduction

Cell invasion is an active process involving dynamic remodeling of the actin cytoskeleton and is a critical step for tumor metastasis, which occurs in 90% of cancer-related human deaths. However, the genetic changes that cause noninvasive tumors to become metastatic are not well understood. A stable epithelial architecture is thought to limit cell proliferation and cell invasion (Dow and Humbert, 2007; Wodarz and Nathke, 2007). Several key

*Correspondence: gmardon@bcm.edu, Tel: (713) 798-8731, Fax: (713) 798-3359.

Conflict of interest
The authors declare no conflict of interest.

molecules have been identified that are required to establish and maintain epithelial integrity, namely the Scribble complex (Scribble (Scrib)/Discs Large (Dlg)/Lethal giant larvae (Lgl)), the Par complex (Par3/Par6/atypical protein kinase C), and the Crumbs complex (Crumbs/Stardust/Patj) (Bilder, 2004). Among these, *scrib*, *dlg*, and *lgl* have been identified as "neoplastic" tumor suppressors, whose loss cause tissue overgrowth accompanied by disruptions in cellular architecture and differentiation (Bilder, 2004). However, clones of *scrib*, *dlg*, or *lgl* survive poorly when surrounded by wild-type cells and are eliminated by cell apoptosis (Agrawal *et al*., 1995; Brumby and Richardson, 2003; Woods and Bryant, 1991). This phenomenon is reminiscent of the multi-gene requirement for a normal cell to become tumorigenic and progress to malignancy (Hanahan and Weinberg, 2000; Land *et al*., 1983). *Drosophila* imaginal discs have become a powerful system to study the effects of multiple genetic changes on discrete populations of cells immediately adjacent to wild-type neighboring cells, which closely resembles the clonal nature of human cancer.

Protein Phosphatase 1 (PP1) is a member of one of the major classes of serine/threonine protein phosphatases, which consists of a catalytic subunit and various regulatory subunits that target the complex to specific locations and regulate substrate specificity (Ceulemans and Bollen, 2004). *PP1* expression is reported to be significantly lower in some human cancer cells (www.oncomine.org) and human PP1 interacts with breast cancer susceptibility protein BRCA1 (Winter *et al*., 2007). Additionally, the PP1 inhibitor okadaic acid has been reported to act as a tumor promoter and can increase migration and invasion of non-metastatic LLC-C8 cells (Suganuma *et al*., 1988; Young, 1997), indicating that loss of *PP1* may contribute to tumor formation and metastasis. However, genetic studies of *PP1* function in vivo have been complicated by the presence of multiple homologs and its involvement in a wide range of cellular processes in most organisms. Therefore, PP1 regulatory subunits can provide a key to understanding the role of PP1 in tumor growth and metastasis.

Sds22 is a conserved, leucine-rich repeat protein first identified as a regulatory subunit of PP1 that is required for the completion of mitosis in yeast (Ohkura and Yanagida, 1991). Recently, one group identified *Drosophila sds22* as a regulator of epithelial polarity (Grusche *et al*., 2009). In this report, we show that, in addition to its role in cell polarity, *sds22* is critical for maintaining epithelial integrity, and that without *sds22* cells become invasive and tumorigenic. Furthermore, *sds22* overexpression can largely suppress the tumorigenic growth of $Ras^{V12}scrib^{-/-}$ cells. Finally, we show that one potential mechanism by which *sds22* prevents cell invasion and metastasis is through inhibition of myosin II and JNK activity downstream of PP1. Together, these results highlight the importance of *sds22* as a novel member of the neoplastic tumor suppressor gene class that links changes in epithelial integrity with signaling pathways driving tumor metastasis.

Results

sds22 behaves as a new potential *Drosophila* tumor suppressor gene

A previous study showed that *sds22* is important for epithelial cell shape and polarity (Grusche *et al*., 2009). Given that loss of cell polarity often synergizes with activated Ras (Ras^{V12}) to induce tumor growth and invasion as seen in *scrib/dlg/lgl* mutants (Brumby and Richardson, 2003; Pagliarini and Xu, 2003), we first tested whether loss of *sds22* will have a similar effect. We generated null alleles of *sds22* by imprecise excision of a nearby P-element insertion (*P {EPgy2} EY06161*) in *Drosophila*, which also deleted another gene named CREG (cellular repressor of E1A-stimulated genes) (Figure S1A). The lethality and mutant phenotypes can be fully rescued by a genomic rescue construct and a *UAS-sds22* transgene, suggesting that *sds22* is the gene responsible for the observed phenotypes (Figure S1C–E). *sds22* homozygotes die at or prior to the first larva instar. To test whether loss of

sds22 promotes tumor growth and metastasis of Ras^{V12} expressing cells, we expressed Ras^{V12} in *sds22* mutant cells using the *eyFLP/*MARCM system, in which 30% of the eye is typically composed of mutant tissue (Lee and Luo, 2001). Consistent with previous reports, Ras^{V12} overexpression alone induces benign overgrowth but cells never invade into the nearby ventral nerve cord (VNC) or other tissues (Figure 1A,D). When Ras^{V12} overexpression is combined with homozygous loss of *sds22*, such animals can grow as larvae for up to 15 days after egg laying (AEL) and die prior to pupation or as early pupae (wild-type animals normally pupate 5 days AEL at 25°C). In contrast, animals expressing Ras^{V12} alone can only grow as larvae for up to 9 days AEL and then die as early pupae. At 7 days AEL, we observe extensive hyperproliferation in eye discs of $Ras^{V12}sds22^{-/-}$ animals (Figure 1B,H) but GFP-positive cells are seen in the VNC at only low frequency (Figure 1E, white arrow). At 15 days AEL we find significant numbers of ectopic GFP-positive cells spreading from a primary tumor in the brain into the VNC (Figure 1F). In addition, as $Ras^{V12}sds22^{-/-}$ tumors grow, the two eye-antennal discs appear to fuse into one large mass (Figure 1I). Together, these results suggest that loss of *sds22* can cooperate with Ras^{V12} to promote tumor growth and invasive behavior in a time-dependent manner.

Next, we asked whether the *sds22* mutation alone is sufficient to cause tumor growth or metastasis. Similar to cells mutant for the neoplastic tumor suppressor genes *scrib*, *dlg* or *lgl*, we find that *sds22* mutant clones are more sensitive to cell competition, exhibit cell apoptosis, and do not over proliferate or metastasize (Figure S2A–G and S3A). The role of Ras signaling in promoting cell survival has been well documented (Bonni *et al*., 1999; Kurada and White, 1998). To test whether the cooperative effect between loss of *sds22* and *Ras* overexpression is linked to cell survival, we coexpressed the baculovirus caspase inhibitor p35 in *sds22* mutant cells using the *eyFLP/*MARCM system to block cell death. Interestingly, these 'undead' cells (Martin *et al*., 2009) induce both cell-autonomous and non-cell autonomous cellular proliferation and result in a massively overgrown and folded eye disc and enlarged tumor-like adult eyes (Figure S3C–C‴), suggesting that loss of *sds22* confers tumor growth when cell death is inhibited. Overexpression of *p35* alone does not cause any obvious growth defects (Figure S3B). However, we do not find GFP-labeled mutant cells outside of the eye-antennal disc/optic lobe region (data not shown), suggesting that blocking cell death is not sufficient to promote metastasis of $sds22^{-/-}$ cells. Combined with the overgrowth phenotype in cooperation with oncogenic Ras, these results suggest that *sds22* mutant cells induce uncontrolled proliferation when combined with a second genetic change or "hit" that promotes cell survival. Given that tumor suppressor mutations often require a second hit to manifest their full phenotypes, these data suggest that *sds22* is a new *Drosophila* tumor suppressor gene.

To further investigate the possible contribution of *sds22* to tumor suppression, we next tested if *sds22* gain-of-function is capable of suppressing tumor growth using the previously established *Drosophila* tumor model $Ras^{V12}scrib^{-/-}$ (Pagliarini and Xu, 2003). Coexpression of Ras^{V12} in *scrib* mutant cells using the *eyFLP/*MARCM system induces strong tumor growth at 7 days AEL (Figure 2A). $Ras^{V12}scrib^{-/-}$ animals keep growing as larvae until 13 days AEL and die before pupation. We find that coexpression of *sds22* strongly suppresses the tumor growth phenotype in all clones observed at 7 days AEL compared to $Ras^{V12}scrib^{-/-}$ alone (Figure 2A,B). Most of these animals can pupate but die as early pupae, while $Ras^{V12}scrib^{-/-}$ animals rarely pupate. These results suggest that overexpression of *sds22* can suppress the tumor-like growthof $Ras^{V12}scrib^{-/-}$ cells. To determine the mechanism by which overexpression of *sds22* activity suppresses $Ras^{V12}scrib^{-/-}$ overproliferation, we tested if *sds22* overexpression can suppress Ras^{V12} or *scrib* phenotypes individually. We observe strong suppression of *scrib* phenotypes in both larval and adult stages by overexpression of *sds22* in *scrib* mutant eye discs (Figure 2C,D). However, overexpression of *sds22* does not suppress the enlarged eye phenotype caused by

overexpression of Ras^{V12} using *ey-GAL4* (Figure 2E–H). Thus, we conclude that *sds22* can suppress tumor growth in part through its interaction with the cell polarity gene *scrib*.

Loss of *sds22* leads to cell invasive behavior

The metastatic capability of $Ras^{V12}sds22^{-/-}$ cells but not Ras^{V12} alone may result from a potential acquired role of *sds22* in preventing cellular invasion. To test this possibility, we used *patched-GAL4* (*ptc-GAL4*)*/UAS-GFP* system to knock down *sds22* using RNAi in a defined region along the anterior/posterior (A/P) compartment boundary of the wing disc, a well used system to study cell migratory behavior in *Drosophila* (Singh *et al.*, 2010; Speck *et al.*, 2003; Srivastava *et al.*, 2007; Vidal *et al.*, 2006). Compared to controls where GFP-marked wild-type cells are localized within a straight stripe (Figure 3A,B), GFP-positive *sds22* deficient cells are basally extruded and migrate away from the *ptc-GAL4* expression domain into the posterior compartment (Figure 3C,D), resulting in an abnormal apical folding of the disc epithelium along the A-P boundary (Figure 6A,B). The A-P compartment boundary remains relatively smooth and regular based on expression of the anterior compartment-specific marker Cubitus interruptus (Ci) (Figure 3C″), indicating that the invasion-like behavior of $sds22^{-/-}$ cells is unlikely to result from disruption of AP compartmentalization.

To test whether the invasion-like phenotype caused by loss of *sds22* is specific to the wing epithelium, we generated *sds22* mutant cells using the *eyFLPcl* technique, which removes ~90% of gene function in the eye disc (Newsome *et al.*, 2000). We find that loss of *sds22*causes severely reduced and disorganized photoreceptor differentiation (Figure 4A,B). Additionally, we find ectopic neurons in the optic stalk (Figure 4B′, green arrow), where they are normally never seen (Figure 4A′, green arrow). This invasion-like phenotype is also observed in *sds22* mitotic clones near the posterior margin of the eye disc (Figure S4B). To test whether these ectopic cells are *sds22* mutant or wild-type, we used the *hsFLP*/MARCM technique to positively mark mutant cells with GFP. We find that the Elav-positive neurons in the optic stalk are also GFP-positive (Figure S4C, white arrows), suggesting that *sds22* mutant cells are migrating away from the eye disc. In addition to photoreceptor cells, we also find undifferentiated cells and cone cells in the eye disc are mislocalized in the optic stalk (data not shown), indicating that the migratory behavior is not simply due to photoreceptor axon extension. Another possibility is that the basal migration by *sds22* mutant cells might be a secondary consequence of cell death. To test this, we blocked cell death by overexpression of *p35* in *sds22* mutant cells. Elav-positive mutant neurons are still mislocalized in the optic stalk (Figure S4D″, white arrow), indicating that cell invasion is not a secondary consequence of cell death induced by loss of *sds22*. Together, these results suggest that *sds22* is required for maintaining proper cellular position in both the wing and eye disc.

sds22 is an essential regulator of PP1

Sds22 physically binds to Protein Phosphatase 1 (PP1) and regulates PP1 activity in yeast and mammalian cells (Ceulemans *et al*., 2002; MacKelvie *et al*., 1995; Stone *et al*., 1993). Binding of the *Drosophila* homolog of Sds22 to PP1 subunits has also been confirmed in a yeast two-hybrid system and *Drosophila* S2 cells (Bennett *et al*., 2006; Grusche *et al*., 2009). However, the functional significance of this interaction has not been studied in vivo and the role of *PP1* in epithelial integrity and cellular invasion is not clear. To explore the mechanism of how loss of *sds22* induces cell invasion-like behavior, we first asked whether loss of PP1 activity causes a similar phenotype as loss of *sds22*. *Drosophila* has four PP1 isoforms, named after theirsubtype and chromosome location: *PP1β9C*, *PP1α13C*, *PP1α87B*, and *PP1α96A* (Dombradi *et al*., 1990; Dombradi *et al*., 1993). Of these, *PP1α13C* and *PP1α96A* are not essential based on loss-of-function studies (Kirchner *et al*.,

2007a; Smith, 1999) and therefore were not included in this study. We find that loss of *PP1α87B* or *PP1β9C* share many features with loss of *sds22*, including loss of tissue architecture and differentiation, increased cell death and cell invasive behavior (Figure 4C–F). Since loss of *sds22* phenotypes in yeast can be suppressed by high dosage of *PP1* (Ohkura and Yanagida, 1991), we tested whether a similar relationship exists in *Drosophila*. Strikingly, overexpression of *PP1β9C*, but not *PP1α87B*, can significantly suppress *sds22* phenotypes (Figure 4G–J). Overexpression of individual PP1 isoforms alone does not cause an obvious phenotype (data not shown). Together, these results suggest *sds22* functions as an essential positive regulator of PP1 to maintain epithelial organization and to block cell invasion.

Myosin II activity is responsible for changes caused by loss of *sds22*

Nonmuscle myosin II (referred to as myosin II hereafter) is an actin-based motor protein complex which plays a crucial role in cytoskeleton and tissue organization (Vicente-Manzanares *et al*., 2009). The myosin II regulatory light chain Spaghetti Squash (Sqh) is a direct target of PP1β9C and dephosphorylation of Sqh inactivates Myosin II (Vereshchagina *et al*., 2004). Phosphorylation of Sqh (p-Sqh) is increased in *sds22* mutant follicle cells (Grusche *et al*., 2009), suggesting that Sqh hyperphosphorylation may play a role in mediating phenotypes caused by loss of *sds22*. To test this hypothesis, we first ectopically expressed a phosphomimetic (activated) form of Sqh (*UAS-sqh*^{DD}) in the eye disc using either the *FLPout* technique or *ey-GAL*. In each case, neurons expressing activated Sqh become mislocalized in the optic stalk (Figure 5A,5I), closely phenocopying *sds22*-mediated cell migratory behavior. In addition, knockdown of myosin II activity by coexpression of an RNAi construct against the myosin IIheavy chain (*zip*) or the regulatory light chain (*sqh*) in *sds22* mutant cells suppresses the *sds22* migratory behavior (Figure 5D′,E′,F′). Moreover, reducing myosin II activity can largely rescue the cell morphology defects of *sds22* mutant cells (Figure 5E″,F″). Knockdown of *zip* or *sqh* alone does not cause any invasion-like phenotype (Figure 5B and data not shown). Taken together, these results suggest that myosin II is essential for *sds22*-mediated cell morphology defects and cell invasion behavior. Interestingly, the phenotypes resulting from myosin II hyperactivity are less severe than those caused by knockdown of either *sds22* or *PP1* (Figure 5G–I), raising the possibility that Sds22/PP1 regulates additional substrates other than Sqh.

JNK signaling is required for loss of *sds22*-mediated cell invasion and apoptosis

The Jun N-terminal kinase (JNK) signaling pathway is an important mediator of tumor invasion (Huang *et al*., 2003; Igaki *et al*., 2006; Uhlirova *et al*., 2005). In addition, activated JNK signaling induces cell apoptosis (Behrens *et al*., 1999). Since loss of *sds22* causes cell invasion and increased cell death, it seems likely that modulation of JNK pathway activity is involved in these phenotypes. To test this hypothesis, we examined transcription levels of *puc*, which encodes a JNK-specific phosphatase and acts as both a downstream target and a feedback inhibitor of the JNK signaling pathway (Martin-Blanco *et al*., 1998; McEwen and Peifer, 2005). Consistent with our hypothesis, *puc-lacZ* reporter expression is increased in *sds22* deficient migrating cells (Figure 6B). Loss of *PP1β* also increases *puc-lacZ* expression (Figure 6C), suggesting an increase in JNK-dependent transcription in *sds22* deficient cells is likely through regulation of PP1 activity by *sds22*. Next, we tested whether active JNK is responsible for the changes observed in *sds22* mutant cells. Increasing JNK signaling alone by overexpression of *eiger* (a ligand for the *Drosophila* JNK pathway) using *ptc-GAL4* is sufficient to cause massive cell migration and cell death (Figure S5C). Importantly, blocking JNK activity by overexpression of *puc* (a JNK activity inhibitor) in *sds22* mutant cells suppresses both cell migration and cell death caused by loss of *sds22* (Figure 7C,D and S5D–F). Overexpression of *puc* alone does not causeany obvious defects in the cytoskeleton or cell invasion (Figure S5G). Finally, blocking JNK activity also fully

suppresses tumor growth and metastasis of $Ras^{V12}sds22^{-/-}$ cells (Figure S6A–D). Collectively, these results suggest that increased JNK signaling plays a substantial role in cell invasion and cell death induced by loss of *sds22*.

Preventing basement membrane degradation suppresses invasiveness of *sds22* mutant cells

JNK functions in part by modulating expression of Matrix metalloprotease 1 (MMP1) to promote tumor cell motility (Jasper *et al*., 2001; Uhlirova and Bohmann, 2006). MMP1 is essential for degradation of the basement membrane (BM) (Deryugina and Quigley, 2006), and is therefore required for metastatic potential of *Drosophila* tumors (Beaucher et al., 2007; Uhlirova and Bohmann, 2006). Consistent with this view, we find dramatically increased expression of MMP1 in both *sds22* and *PP1* mutant eye discs compared to controls (Figure 6E,F). To test whether MMPs play a role in *sds22*-mediated cell invasion, we blocked MMP function in *sds22* mutant clones by ectopic expression of *Timp*, which encodes a *Drosophila* homolog of the Tissue inhibitor of metalloproteases (Llano *et al*., 2000; Page-McCaw *et al*., 2003; Pohar *et al*., 1999). We observe that overexpression of *Timp* using *ptc-GAL4* strongly suppresses the invasive behavior of *sds22* deficient cells in the wing disc (Figure 7E,F, green arrowhead), while overexpression of *Timp* alone causes no obvious defects (data not shown). These data suggest that MMP activity is critical for the cell invasive behavior caused by loss of *sds22*. In addition, we find that epithelial organization defects, including an abnormal apical folding along the A-P boundary of the wing disc, are not rescued by overexpression of either *puc* or *Timp* (Figure 7D″,F″, yellow arrowheads), suggesting that hyperactivity of myosin II may be sufficient to mediate this epithelial integrity defect.

Discussion

Stable epithelial integrity is required for normal tissue morphogenesis during development, and its loss is often associated with cancer. The importance of *sds22* in regulating epithelial morphology has been recently reported (Grusche et al., 2009). However, the detailed mechanism of *sds22* function and its role in tumor suppression have not been studied. By generating new, null alleles of *sds22* in *Drosophila*, we show for the first time that *sds22* is a new potential tumor suppressor gene that plays a key role in the metastatic process. Consistent with the work of Grusche et al., our results show that *sds22* mutant cells lose epithelial organization, fail to differentiate normally, and undergo cell death. Beyond this, we show that *sds22* mutant cells become invasive and migrate into neighboring regions, likely by increasing Matrix metalloprotease 1 (MMP1) secretion to degrade the basement membrane. Importantly, *sds22* mutant cells undergo uncontrolled proliferation when cell death is blocked or in cooperation with activated Ras. Conversely, overexpression of *sds22* can substantially delay tumor growth of $Ras^{V12}scrib^{-/-}$ cells and suppress the *scrib* phenotype in vivo, consistent with *sds22* functioning as a tumor suppressor gene. Finally, our genetic evidence leads us to propose a novel model in which *sds22* functions as an essential positive regulator of PP1 to restrict myosin II and JNK activity, thereby maintaining epithelial integrity and preventing proliferation and metastasis (see model in Figure 7G), which provides significant new mechanistic insights into tumor suppressor pathways.

Tumor suppressive properties of *sds22* mutant cells in epithelial tissues

Most human tumors are derived from epithelial tissues and loss of epithelial integrity has been linked to tumor growth and invasion (Brumby and Richardson, 2003; Igaki *et al*., 2006; Pagliarini and Xu, 2003). Here, we provide evidence that *sds22* is a regulator of epithelial integrity and cell invasion, two key characteristics of malignant epithelial cells (Hanahan

and Weinberg, 2000). We have considered the possibility that the invasion-like behavior of *sds22*$^{-/-}$ cells might be secondary to defects in cell death or cell adhesion. However, not all invasive *sds22*$^{-/-}$ cells are Caspase-3 positive and blocking cell death does not suppress cell invasion behavior. Additionally, we find loss of *sds22* always causes directional migration, while defects in cell adhesion often cause cells to disperse into surrounding wild type cells (Knox and Brown, 2002). Moreover, loss of *sds22* is sufficient to induce metastatic behavior of *Ras*^{V12} cells, while loss of cell adhesion molecules, such as E-cadherin, does not (Pagliarini and Xu, 2003). Finally, loss of *sds22* can induce MMP1 secretion downstream of JNK signaling, which is known to be activated by invading cells. Taken together, these data support the view that *sds22*$^{-/-}$ cells actively invade surrounding tissue.

Why does loss of *sds22* alone not cause tumor-like growth? In human cancer, it is rare that mutation of a single gene is sufficient to cause malignant transformation. Instead, multiple mutations are most often required for tumorigenesis (Brumby and Richardson, 2005; Land *et al*., 1983). Similar to the tumor suppressor *scrib*, loss of *sds22* induces massive cell death, presumably as a result of stresses induced by loss of epithelial integrity. However, when cell death is blocked by expression of the caspase inhibitors p35, *sds22*$^{-/-}$ cells can grow to form large, tumor-like masses. Additionally, loss of *sds22* in combination with expression of oncogenic Ras promotes tumor growth and metastasis, similar to studies of other tumor suppressors involved in maintenance of cell polarity (Pagliarini and Xu, 2003). Interestingly, blocking cell death in *sds22* mutant cells is not sufficient to induce tumor metastasis, suggesting that there must be an additional mechanism of Ras function other than promoting cell survival to account for tumor invasion.

A new role for PP1 in epithelial organization and cell invasion through regulation of myosin II and JNK

Both *Drosophila* and humans have multiple genes encoding PP1c isoforms, which has complicated analysis of their biological roles in vivo. In this study, we provide the first in vivo evidence that *PP1* plays essential roles in controlling epithelial organization and cell invasion. Our studies suggest that *sds22* functions as a key regulatory subunit of PP1 to inhibit myosin II and JNK signaling. In addition to the previously identified target myosin II (Grusche *et al*., 2009), we find that JNK signaling is also regulated by *sds22/PP1*. How *sds22* regulates JNK signaling, which mediates both cell invasion and cell apoptosis, remains unclear. The fact that not all *sds22* deficient cells induce active JNK indicates that *sds22/PP1* may regulate JNK activity indirectly through regulation of upstream components. Genetic studies suggest that *Drosophila PP1β* can regulate JNK through myosin II (Kirchner *et al.*, 2007b). However, blocking myosin II activity in our study does not abolish the *sds22/PP1*-mediated JNK activation (data not shown). Alternatively, the JNK pathway can be activated by disruption of cell polarity genes (Brumby and Richardson, 2003; Ryoo *et al.*, 2004; Zhu *et al.*, 2010), suggesting that JNK could be a common downstream signal induced by the absence of these tumor suppressors. The role of cell polarity genes in mediating JNK activation downstream of *sds22/PP1* will require further investigation.

Relationship between Sds22/PP1 and cell polarity genes

Although the cell invasion and death phenotypes caused by loss of *sds22* can be fully suppressed by reducing myosin II and JNK activity, epithelial defects are not fully rescued, suggesting that additional targets of the Sds22/PP1 complex might be involved. Phosphorylation of cell polarity regulators, including Baz and Lgl, must be tightly regulated for their normal subcellular localization and function (Betschinger *et al.*, 2003; Morais-de-Sa *et al.*, 2010). Although much is known regarding the roles of their kinases such as Par-1 and aPKC, the mechanism of their dephosphorylation is unclear. Recently, *sds22* was identified in a geneticinteraction screen with *Baz* (Shao *et al*., 2010), a key regulator of

apical membrane polarity and a substrate of PP1 in mouse cell culture (Traweger *et al*., 2008), suggesting that *sds22/PP1* may act directly on critical components of the cell polarity machinery to maintain epithelial integrity and prevent metastasis. Consistent with this interpretation, we find that overexpression of *sds22* can largely suppress the loss-of-function phenotypes of the cell polarity gene *scrib*. Further research will be necessary to clarify the mechanism of the interplay between Sds22/PP1 and cell polarity genes.

Sds22/PP1 function in mammals

The proteins Sds22, PP1, and components of myosin II and the JNK signaling pathway are highly conserved between *Drosophila* and humans. This raises the possibility that human *Sds22* may play a role in regulating PP1 to maintain proper epithelial integrity and prevent cell invasion via a mechanism similar to that reported in *Drosophila*. Indeed, the human *sds22* homolog, *PPP1R7,* also regulates cell shape and myosin II light chain phosphorylation (Grusche *et al*., 2009). In support of a tumor suppressive role for *PPP1R7* in cancer, a survey of the Turmorscape portal (http://www.broadinstitute.org/tumorscape/) for copy number alterations in cancer (Beroukhim *et al.*, 2010) shows that *PPP1R7* (2q37.3), is frequently deleted in six cancer subtypes that include breast, ovarian, and melanoma among others (Figure S7A). This finding is consistent with published reports indicating *PPP1R7* deletion in oral and cervical cancer (Cengiz *et al.*, 2007; Narayan *et al.*, 2003). Consistent with its genomic loss, *PPP1R7* RNA expression is also significantly down-regulated in multiple cancer types (Rhodes et al., 2004). Among those cancers is melanoma, where *PPP1R7* expression is down-regulated in primary tumors versus normal skin and benign nevi (Talantov et al., 2005) and in melanoma metastases versus primary tumor specimens (Kabbarah *et al.*, 2010) (Figure S7B). Collectively, these findings support a role for *PPP1R7* in tumor suppression in mammals and emphasize the importance of epithelial regulators in tumor progression.

In conclusion, the data presented here add new information about the role of *sds22* during normal epithelial tissue organization and tumor cell invasion. Our studies show that the interaction of Sds22 with PP1 regulates a subset of the proteins normally controlled by PP1 activity and affects signaling pathways involved in apoptosis, cell migration, and cytoskeleton control, and whose misregulation leads to enhanced invasive behavior and transforms cells from a nonmetastatic to a metastatic state. Importantly, we also find that *sds22* interacts with the known neoplastic tumor suppressor *scrib*, and can cooperate with activated Ras to promote tissue neoplasia and metastasis. Together, our results raise the interesting possibility that dephosphorylation of key molecules that normally control cell polarity and cell migration through *sds22/PP1* activity could be a previously unrecognized tumor suppression mechanism.

Experimental Procedures

Drosophila Stocks

Fly cultures were raised at 25°C using standard media. The following stocks were used: *P{EPgy2}EY06161*, *PP1a-87B*^{87Bg3}, *UAS-PP1a-87B*, and *UAS-PP1β-9C* (Bloomington Stock Center), *sds22-RNAi*, *PP1β9C-RNAi*, *zip-RNAi* (VDRC), *UAS-sqh*^{DD} (Yasuyoshi Nishida), *UAS-eiger* and *UAS-Ras*^{V12} (Tian Xu), *UAS-puc*, and *puc-lacZ* (Andreas Bergmann), *scrib*^{2} (Georg Halder), *UAS-Timp* (Dirk Bohmann). *sds22*$^{Δ1.8}$, *sds22*$^{Δ1.83}$, *sds22*$^{Δ2.2}$, *sds22*$^{Δ2.3}$ and *sds22*$^{Δ2.8}$ were derived from imprecise excision of the P-element *P{EPgy2}EY06161* using *Δ2–3* transposase following conventional methods. All these alleles fail to complement each other and yield the same lethal phase and adult eye phenotypes described in this study.

Generation of Rescue Constructs

For rescue experiments, *UAS-sds22* contains a full-length cDNA from EST clone *GH07711* between the EcoRI and XhoI sites of *pUAST-attB*. An *sds22* construct that contains only the *sds22* genomic locus rescues all known *sds22* mutant phenotypes. Details of this construct are available upon request.

Clonal Analysis

Mitotic or RNAi *FLPout* clones were generated by the FRT/FLP technique (Xu and Rubin, 1993) by applying a 1 hr heat shock (37°C) to induce *hs-FLP* 48 hr AEL. The *eyFLP/* MARCM system (Lee and Luo, 2001) was used to induce clones positively marked by GFP in eye/antennal discs.

Immunohistochemistry

Antibody stainings of imaginal discs were performed as previously described (Pepple *et al*., 2008). The following antibodies were used: rat anti-Elav (1:400, DSHB), chicken-anti-GFP (1:1000, Sigma), rat anti-DE cadherin (1:50, DSHB), Mouse anti-MMP1 (1:150, from Andreas Bergmann), rabbit anti-β-Galactosidase (1:1000, Cappel), mouse anti-Dlg (1:100, DSHB), and Rabbit anti-Caspase-3 (1:100, Cell Signaling), rhodamine-conjugated phalloidin (1:50, Molecular Probes). Secondary antibodies were anti-rat-Alexa 488, anti-guinea pig-Cy3, anti-chicken-Alexa 488, anti-rat-Cy3, anti-mouse-Cy3, anti-rabbit-Cy3 (1:1000 each, Molecular Probes), and anti-mouse-Cy5 (1:1000, Jackson Immunochemicals). Images were captured with a Zeiss LSM 510 confocal microscope and processed with ImageJ and Adobe Photoshop software. The images of compared genotypes are at the same magnification.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Andreas Bergmann, Yasuyoshi Nishida, Tian Xu, the Bloomington Stock Center, VDRC, and the DSHB for stocks and antibodies. We especially thank Georg Halder, Rui Chen, Mardelle Atkins, Umesh Karandikar, and Nicholas Justice for helpful comments on the manuscript. This work was supported by the National Eye Institute (R01 EY011232 [G.M.] and EY016853 [R.C.]) and the Retina Research Foundation (G.M.).

References

Agrawal N, Kango M, Mishra A, Sinha P. Neoplastic transformation and aberrant cell-cell interactions in genetic mosaics of lethal(2)giant larvae (lgl), a tumor suppressor gene of Drosophila. Dev Biol. 1995; 172:218–29. [PubMed: 7589802]

Beaucher M, Hersperger E, Page-McCaw A, Shearn A. Metastatic ability of Drosophila tumors depends on MMP activity. Dev Biol. 2007; 303:625–34. [PubMed: 17239363]

Behrens A, Sibilia M, Wagner EF. Amino-terminal phosphorylation of c-Jun regulates stress-induced apoptosis and cellular proliferation. Nat Genet. 1999; 21:326–9. [PubMed: 10080190]

Bennett D, Lyulcheva E, Alphey L, Hawcroft G. Towards a comprehensive analysis of the protein phosphatase 1 interactome in Drosophila. J Mol Biol. 2006; 364:196–212. [PubMed: 17007873]

Beroukhim R, Mermel CH, Porter D, Wei G, Raychaudhuri S, Donovan J, et al. The landscape of somatic copy-number alteration across human cancers. Nature. 2010; 463:899–905. [PubMed: 20164920]

Betschinger J, Mechtler K, Knoblich JA. The Par complex directs asymmetric cell division by phosphorylating the cytoskeletal protein Lgl. Nature. 2003; 422:326–30. [PubMed: 12629552]

Bilder D. Epithelial polarity and proliferation control: links from the Drosophila neoplastic tumor suppressors. Genes Dev. 2004; 18:1909–25. [PubMed: 15314019]

Bonni A, Brunet A, West AE, Datta SR, Takasu MA, Greenberg ME. Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and -independent mechanisms. Science. 1999; 286:1358–62. [PubMed: 10558990]

Brumby AM, Richardson HE. scribble mutants cooperate with oncogenic Ras or Notch to cause neoplastic overgrowth in Drosophila. EMBO J. 2003; 22:5769–79. [PubMed: 14592975]

Brumby AM, Richardson HE. Using Drosophila melanogaster to map human cancer pathways. Nat Rev Cancer. 2005; 5:626–39. [PubMed: 16034367]

Cengiz B, Gunduz M, Nagatsuka H, Beder L, Gunduz E, Tamamura R, et al. Fine deletion mapping of chromosome 2q21–37 shows three preferentially deleted regions in oral cancer. Oral Oncol. 2007; 43:241–7. [PubMed: 16857411]

Ceulemans H, Bollen M. Functional diversity of protein phosphatase-1, a cellular economizer and reset button. Physiol Rev. 2004; 84:1–39. [PubMed: 14715909]

Ceulemans H, Vulsteke V, De Maeyer M, Tatchell K, Stalmans W, Bollen M. Binding of the concave surface of the Sds22 superhelix to the alpha 4/alpha 5/alpha 6-triangle of protein phosphatase-1. J Biol Chem. 2002; 277:47331–7. [PubMed: 12226088]

Deryugina EI, Quigley JP. Matrix metalloproteinases and tumor metastasis. Cancer Metastasis Rev. 2006; 25:9–34. [PubMed: 16680569]

Dombradi V, Axton JM, Brewis ND, da Cruz e Silva EF, Alphey L, Cohen PT. Drosophila contains three genes that encode distinct isoforms of protein phosphatase 1. Eur J Biochem. 1990; 194:739–45. [PubMed: 2176604]

Dombradi V, Mann DJ, Saunders RD, Cohen PT. Cloning of the fourth functional gene for protein phosphatase 1 in Drosophila melanogaster from its chromosomal location. Eur J Biochem. 1993; 212:177–83. [PubMed: 8383037]

Dow LE, Humbert PO. Polarity regulators and the control of epithelial architecture, cell migration, and tumorigenesis. Int Rev Cytol. 2007; 262:253–302. [PubMed: 17631191]

Grusche FA, Hidalgo C, Fletcher G, Sung HH, Sahai E, Thompson BJ. Sds22, a PP1 phosphatase regulatory subunit, regulates epithelial cell polarity and shape [Sds22 in epithelial morphology]. BMC Dev Biol. 2009; 9:14. [PubMed: 19228425]

Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000; 100:57–70. [PubMed: 10647931]

Huang C, Rajfur Z, Borchers C, Schaller MD, Jacobson K. JNK phosphorylates paxillin and regulates cell migration. Nature. 2003; 424:219–23. [PubMed: 12853963]

Igaki T, Pagliarini RA, Xu T. Loss of cell polarity drives tumor growth and invasion through JNK activation in Drosophila. Curr Biol. 2006; 16:1139–46. [PubMed: 16753569]

Jasper H, Benes V, Schwager C, Sauer S, Clauder-Munster S, Ansorge W, et al. The genomic response of the Drosophila embryo to JNK signaling. Dev Cell. 2001; 1:579–86. [PubMed: 11703947]

Kabbarah O, Nogueira C, Feng B, Nazarian RM, Bosenberg M, Wu M, et al. Integrative genome comparison of primary and metastatic melanomas. PLoS One. 2010; 5:e10770. [PubMed: 20520718]

Kirchner J, Gross S, Bennett D, Alphey L. Essential, overlapping and redundant roles of the Drosophila protein phosphatase 1 alpha and 1 beta genes. Genetics. 2007a; 176:273–81. [PubMed: 17513890]

Kirchner J, Gross S, Bennett D, Alphey L. The nonmuscle myosin phosphatase PP1beta (flapwing) negatively regulates Jun N-terminal kinase in wing imaginal discs of Drosophila. Genetics. 2007b; 175:1741–9. [PubMed: 17277363]

Knox AL, Brown NH. Rap1 GTPase regulation of adherens junction positioning and cell adhesion. Science. 2002; 295:1285–8. [PubMed: 11847339]

Kurada P, White K. Ras promotes cell survival in Drosophila by downregulating hid expression. Cell. 1998; 95:319–29. [PubMed: 9814703]

Land H, Parada LF, Weinberg RA. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. Nature. 1983; 304:596–602. [PubMed: 6308472]

Lee T, Luo L. Mosaic analysis with a repressible cell marker (MARCM) for Drosophila neural development. Trends Neurosci. 2001; 24:251–4. [PubMed: 11311363]

Llano E, Pendas AM, Aza-Blanc P, Kornberg TB, Lopez-Otin C. Dm1-MMP, a matrix metalloproteinase from Drosophila with a potential role in extracellular matrix remodeling during neural development. J Biol Chem. 2000; 275:35978–85. [PubMed: 10964925]

MacKelvie SH, Andrews PD, Stark MJ. The Saccharomyces cerevisiae gene SDS22 encodes a potential regulator of the mitotic function of yeast type 1 protein phosphatase. Mol Cell Biol. 1995; 15:3777–85. [PubMed: 7791785]

Martin FA, Perez-Garijo A, Morata G. Apoptosis in Drosophila: compensatory proliferation and undead cells. Int J Dev Biol. 2009; 53:1341–7. [PubMed: 19247932]

Martin-Blanco E, Gampel A, Ring J, Virdee K, Kirov N, Tolkovsky AM, et al. puckered encodes a phosphatase that mediates a feedback loop regulating JNK activity during dorsal closure in Drosophila. Genes Dev. 1998; 12:557–70. [PubMed: 9472024]

McEwen DG, Peifer M. Puckered, a Drosophila MAPK phosphatase, ensures cell viability by antagonizing JNK-induced apoptosis. Development. 2005; 132:3935–46. [PubMed: 16079158]

Morais-de-Sa E, Mirouse V, St Johnston D. aPKC phosphorylation of Bazooka defines the apical/lateral border in Drosophila epithelial cells. Cell. 2010; 141:509–23. [PubMed: 20434988]

Munro E, Nance J, Priess JR. Cortical flows powered by asymmetrical contraction transport PAR proteins to establish and maintain anterior-posterior polarity in the early C. elegans embryo. Dev Cell. 2004; 7:413–24. [PubMed: 15363415]

Narayan G, Pulido HA, Koul S, Lu XY, Harris CP, Yeh YA, et al. Genetic analysis identifies putative tumor suppressor sites at 2q35-q36.1 and 2q36.3-q37.1 involved in cervical cancer progression. Oncogene. 2003; 22:3489–99. [PubMed: 12776201]

Newsome TP, Asling B, Dickson BJ. Analysis of Drosophila photoreceptor axon guidance in eye-specific mosaics. Development. 2000; 127:851–60. [PubMed: 10648243]

Ohkura H, Yanagida M. S. pombe gene sds22+ essential for a midmitotic transition encodes a leucine-rich repeat protein that positively modulates protein phosphatase-1. Cell. 1991; 64:149–57. [PubMed: 1846086]

Page-McCaw A, Serano J, Sante JM, Rubin GM. Drosophila matrix metalloproteinases are required for tissue remodeling, but not embryonic development. Dev Cell. 2003; 4:95–106. [PubMed: 12530966]

Pagliarini RA, Xu T. A genetic screen in Drosophila for metastatic behavior. Science. 2003; 302:1227–31. [PubMed: 14551319]

Pepple KL, Atkins M, Venken K, Wellnitz K, Harding M, Frankfort B, et al. Two-step selection of a single R8 photoreceptor: a bistable loop between senseless and rough locks in R8 fate. Development. 2008; 135:4071–9. [PubMed: 19004852]

Pohar N, Godenschwege TA, Buchner E. Invertebrate tissue inhibitor of metalloproteinase: structure and nested gene organization within the synapsin locus is conserved from Drosophila to human. Genomics. 1999; 57:293–6. [PubMed: 10198170]

Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally R, Ghosh D, et al. ONCOMINE: a cancer microarray database and integrated data-mining platform. Neoplasia. 2004; 6:1–6. [PubMed: 15068665]

Ryoo HD, Gorenc T, Steller H. Apoptotic cells can induce compensatory cell proliferation through the JNK and the Wingless signaling pathways. Dev Cell. 2004; 7:491–501. [PubMed: 15469838]

Shao W, Wu J, Chen J, Lee DM, Tishkina A, Harris TJ. A modifier screen for Bazooka/PAR-3 interacting genes in the Drosophila embryo epithelium. PLoS One. 2010; 5:e9938. [PubMed: 20368978]

Singh J, Aaronson SA, Mlodzik M. Drosophila Abelson kinase mediates cell invasion and proliferation through two distinct MAPK pathways. Oncogene. 2010; 29:4033–45. [PubMed: 20453880]

Smith DP. Drosophila odor receptors revealed. Neuron. 1999; 22:203–4. [PubMed: 10069323]

Speck O, Hughes SC, Noren NK, Kulikauskas RM, Fehon RG. Moesin functions antagonistically to the Rho pathway to maintain epithelial integrity. Nature. 2003; 421:83–7. [PubMed: 12511959]

Srivastava A, Pastor-Pareja JC, Igaki T, Pagliarini R, Xu T. Basement membrane remodeling is essential for Drosophila disc eversion and tumor invasion. Proc Natl Acad Sci U S A. 2007; 104:2721–6. [PubMed: 17301221]

Stone EM, Yamano H, Kinoshita N, Yanagida M. Mitotic regulation of protein phosphatases by the fission yeast sds22 protein. Curr Biol. 1993; 3:13–26. [PubMed: 15335873]

Suganuma M, Fujiki H, Suguri H, Yoshizawa S, Hirota M, Nakayasu M, et al. Okadaic acid: an additional non-phorbol-12-tetradecanoate-13-acetate-type tumor promoter. Proc Natl Acad Sci U S A. 1988; 85:1768–71. [PubMed: 3126494]

Talantov D, Mazumder A, Yu JX, Briggs T, Jiang Y, Backus J, et al. Novel genes associated with malignant melanoma but not benign melanocytic lesions. Clin Cancer Res. 2005; 11:7234–42. [PubMed: 16243793]

Traweger A, Wiggin G, Taylor L, Tate SA, Metalnikov P, Pawson T. Protein phosphatase 1 regulates the phosphorylation state of the polarity scaffold Par-3. Proc Natl Acad Sci U S A. 2008; 105:10402–7. [PubMed: 18641122]

Uhlirova M, Bohmann D. JNK- and Fos-regulated Mmp1 expression cooperates with Ras to induce invasive tumors in Drosophila. EMBO J. 2006; 25:5294–304. [PubMed: 17082773]

Uhlirova M, Jasper H, Bohmann D. Non-cell-autonomous induction of tissue overgrowth by JNK/Ras cooperation in a Drosophila tumor model. Proc Natl Acad Sci U S A. 2005; 102:13123–8. [PubMed: 16150723]

Vereshchagina N, Bennett D, Szoor B, Kirchner J, Gross S, Vissi E, et al. The essential role of PP1beta in Drosophila is to regulate nonmuscle myosin. Mol Biol Cell. 2004; 15:4395–405. [PubMed: 15269282]

Vicente-Manzanares M, Ma X, Adelstein RS, Horwitz AR. Non-muscle myosin II takes centre stage in cell adhesion and migration. Nat Rev Mol Cell Biol. 2009; 10:778–90. [PubMed: 19851336]

Vidal M, Larson DE, Cagan RL. Csk-deficient boundary cells are eliminated from normal Drosophila epithelia by exclusion, migration, and apoptosis. Dev Cell. 2006; 10:33–44. [PubMed: 16399076]

Wagner EF, Nebreda AR. Signal integration by JNK and p38 MAPK pathways in cancer development. Nat Rev Cancer. 2009; 9:537–49. [PubMed: 19629069]

Winter SL, Bosnoyan-Collins L, Pinnaduwage D, Andrulis IL. The interaction of PP1 with BRCA1 and analysis of their expression in breast tumors. BMC Cancer. 2007; 7:85. [PubMed: 17511879]

Wodarz A, Nathke I. Cell polarity in development and cancer. Nat Cell Biol. 2007; 9:1016–24. [PubMed: 17762893]

Woods DF, Bryant PJ. The discs-large tumor suppressor gene of Drosophila encodes a guanylate kinase homolog localized at septate junctions. Cell. 1991; 66:451–64. [PubMed: 1651169]

Xu T, Rubin GM. Analysis of genetic mosaics in developing and adult Drosophila tissues. Development. 1993; 117:1223–37. [PubMed: 8404527]

Young MR. Protein phosphatases-1 and -2A regulate tumor cell migration, invasion and cytoskeletal organization. Adv Exp Med Biol. 1997; 407:311–8. [PubMed: 9321969]

Zhu M, Xin T, Weng S, Gao Y, Zhang Y, Li Q, et al. Activation of JNK signaling links lgl mutations to disruption of the cell polarity and epithelial organization in Drosophila imaginal discs. Cell Res. 2010; 20:242–5. [PubMed: 20066009]

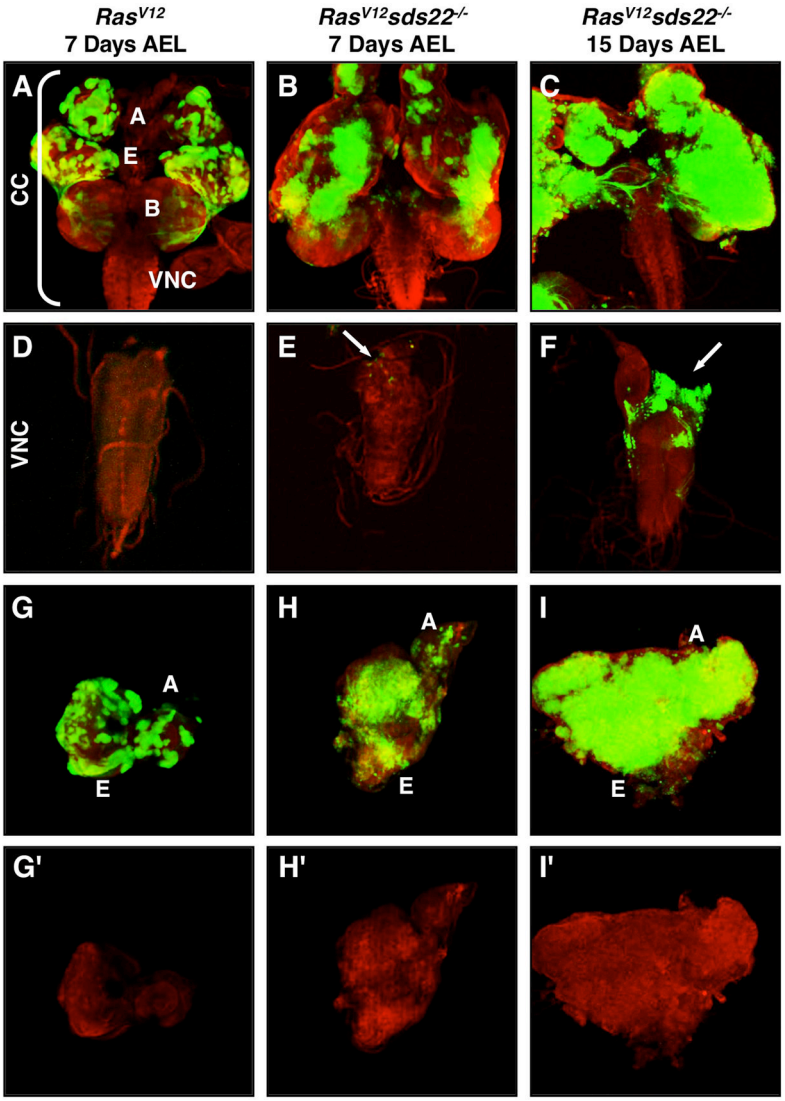

Figure 1. Loss of *sds22* promotes tumor growth and metastasis of Ras^{V12} cells

eyFLP-induced mutant clones (green) of Ras^{V12} cells 7 days after egg laying (AEL) (A,D,G), and $Ras^{V12}sds22^{-/-}$ cells either 7 days AEL (B,E,H) or 15 days AEL (C,F,I) from third-instar larvae are double labeled with GFP and Texas-Red-conjugated phalloidin (red). (A–F) Upper panels show the cephalic complex (CC), including eye (E) and antennal (A) discs, brain (B), and ventral nerve cord (VNC). Lower panels show representative VNCs. $Ras^{V12}sds22^{-/-}$ animals show progressive growth and migration of tumor cells. White arrows indicate ectopic GFP cells in the VNC (E,F), where they are normally absent in Ras^{V12} mutants (D). (G–I) Compared to the control Ras^{V12} eye-antennal disc (G), $Ras^{V12}sds22^{-/-}$ (H,I) mutant discs show strong overproliferation as indicated by the intensity and size of GFP clones and appear to fuse into one large mass at day 15 AEL (I).

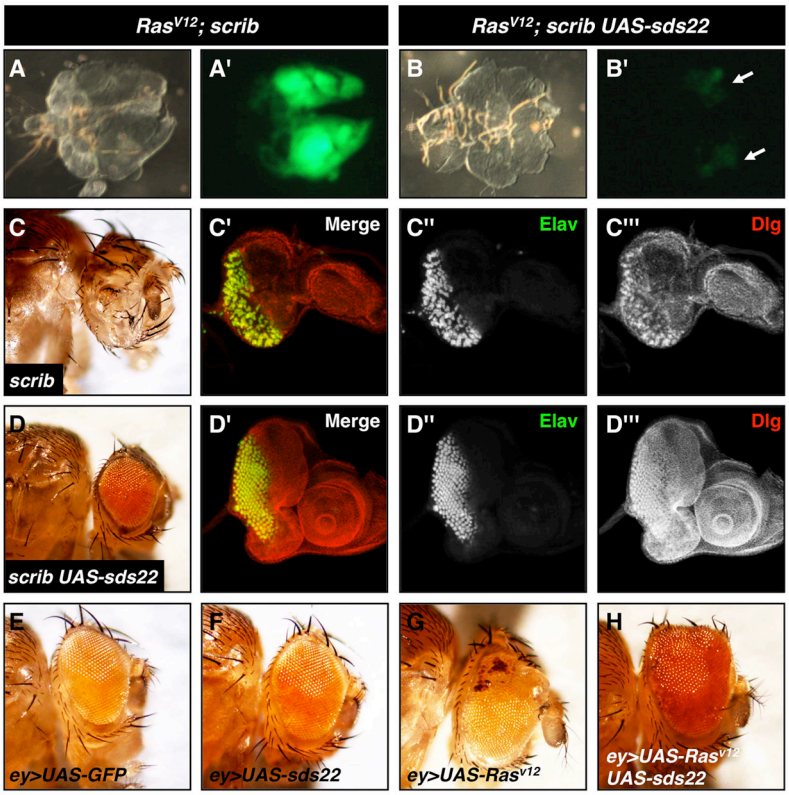

Figure 2. Overexpression of *sds22* suppresses the *Ras^{V12}scrib$^{-/-}$* tumor growth phenotype

(A,B) Cephalic complexes dissected from third instar larvae at 7 days AEL. The same complex is shown as both bright field (left) and fluorescent (right) images. (A–A′) The *Ras^{V12}scrib$^{-/-}$* genotype leads to tumorigenic growth as indicated by the large size of GFP-marked clones. (B–B′) Overexpression of *sds22* in *Ras^{V12}scrib$^{-/-}$* cells substantially suppresses the tumor growth phenotype, indicated by the reduced size and intensity of GFP clones (white arrows indicate the paired eye-antennal discs). (C) Loss of *scrib* generated by *EGUFcl* causes defects in differentiation (Elav, green, C″) and disc morphology (Dlg, red, C‴t) in the third instar eye disc. Most of these animals die as pupae and the few escapers found are eyeless (C). (D) Overexpression of *sds22* suppresses *scrib* mutant defects both in adults (D) and larvae (D′–D‴). (E–H) Light microscopy of adult eyes from animals carrying *ey-GAL4* and: (E) *UAS-GFP*, (F) *UAS-sds22*, (G) *UAS-Ras^{V12}*, and (H) *UAS-Ras^{V12}*, *UAS-sds22*. Compared to the control (E), overexpression of *Ras^{V12}* induces eye overgrowth (G) that is not suppressed by overexpressionof *sds22* (H). Note that overexpression of *sds22* alone does not cause external eye defects (F).

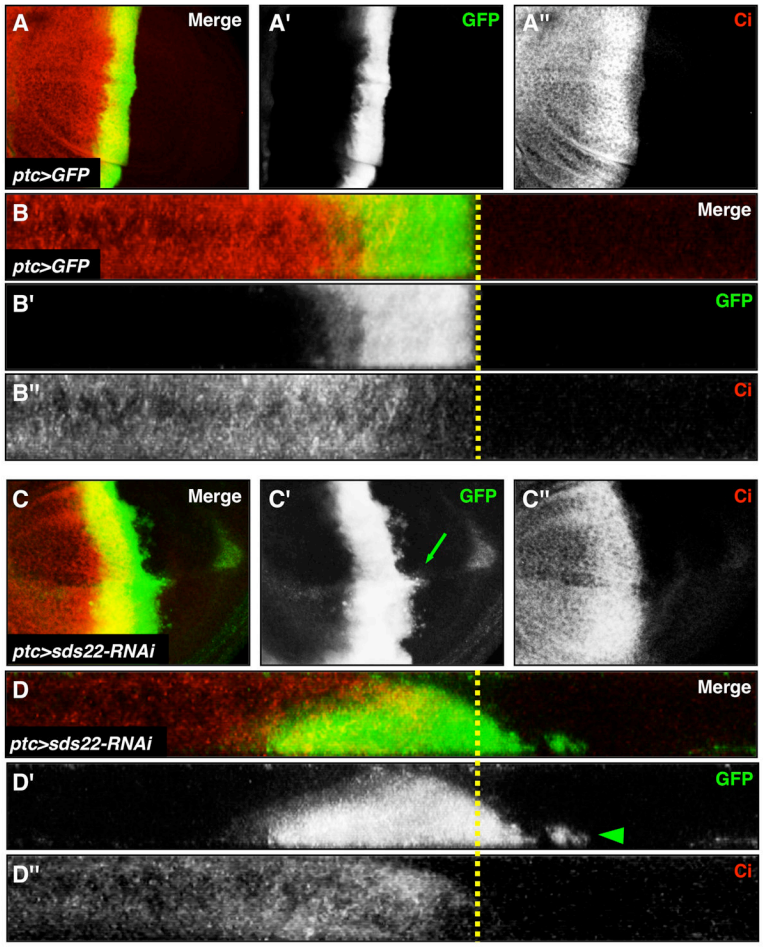

Figure 3. *sds22* deficient cells are basally excluded and migrate toward the posterior compartment

Confocal projections of third instar wing discs expressing either *UAS-GFP* alone (A), or *UAS-GFP* and *sds22-RNAi* (B), both driven by *ptc-GAL4* along the anterior-posterior (A-P) compartment boundary. Here and in all subsequent figures with wing discs, anterior is to the left and dorsal is up. GFP (green) marks mutant cells and Ci (red) marks anterior cells. (A,B) All GFP-marked cells form a cohesive stripe in the *ptc* expression region. The posterior edge of GFP expression is coincident with the low Ci expression, which marks the A-P boundary (indicated by a yellow dash line). (C,D) In contrast to controls, *sds22* deficient cells migrate away from the A-P boundary into the wild-type posterior compartment (green arrow in C′) and are localized basally from X/Z sections (white arrowhead in D′). Note the A-P boundary is straight and smooth, suggesting the A-P compartment may not be disturbed in mutant cells.

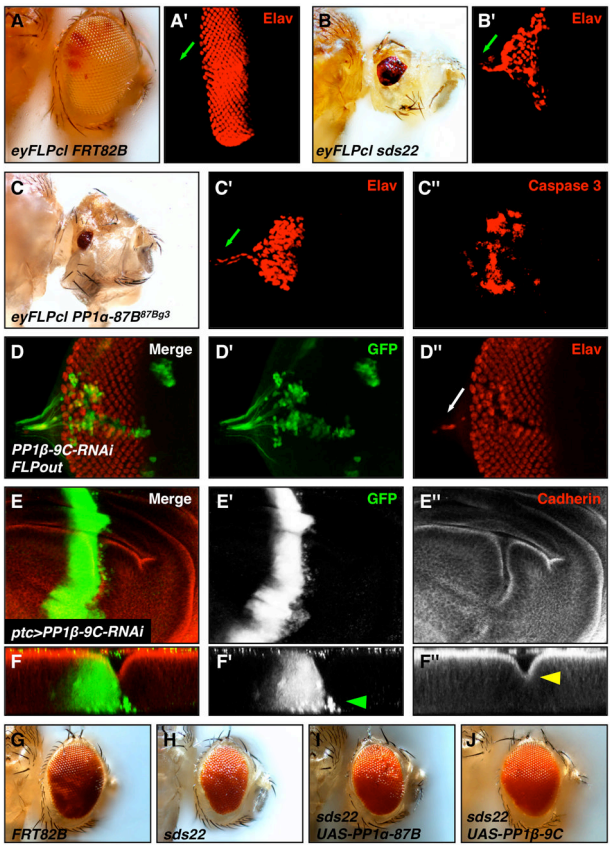

Figure 4. Loss of *PP1* causes similar phenotypes as loss of *sds22*, and overexpression of *PP1β* can suppress *sds22* mutant phenotypes

(A–C) Adult mosaic heads and corresponding third instar eye discs generated by using the *eyFLPcl/FRT* system: (A–A′) *FRT82*, (B–B′) *sds22*, and (C–C″) *PP1α*. Anti-Elav (red) staining reveals photoreceptor cells. Eye morphogenesis is disrupted and eye size is reduced in either *sds22* or *PP1α* mutants compared to controls. Additionally, photoreceptor cells are mislocalized in the optic stalk of mutant discs but not in controls (green arrows in A′,B′,C′). Loss of *PP1α* induces strong cell death as revealed by activated Caspase-3 staining (C″). (D) *PP1β* deficient cells were made using RNAi knockdown and the *FLPout* technique in which mutant cells are positively marked by GFP (green). Mutant Elav-positive cells (red and green) are disorganizedin the clone and are mislocalized in the optic stalk (white arrow in D ″). (E) *PP1β* deficient cells generated using *ptc-GAL4* migrate away from the boundary into the wild-type posterior compartment. X/Z sections from the wing pouch domain show these migrating cells are localized basally (green arrowhead in F′). Note that mutant cells have a disorganized pattern of Cadherin expression and exhibit an abnormal folding (yellow arrowhead in F″). (G–J) Light microscopy of adult mosaic heads generated by the *eyFLP/* MARCM technique: (G) *FRT82B*, (H) $sds22^{e00975}$, (I) $sds22^{e00975}$ *UAS-PP1a-87B*, and (J) $sds22^{e00975}$ *UAS-PP1β -9C*. Note that coexpression of *PP1β -9C*, but not *PP1a-87B*, along with loss of *sds22* results in a largely restored adult eye, suggesting that *PP1β* acts downstream of *sds22*.

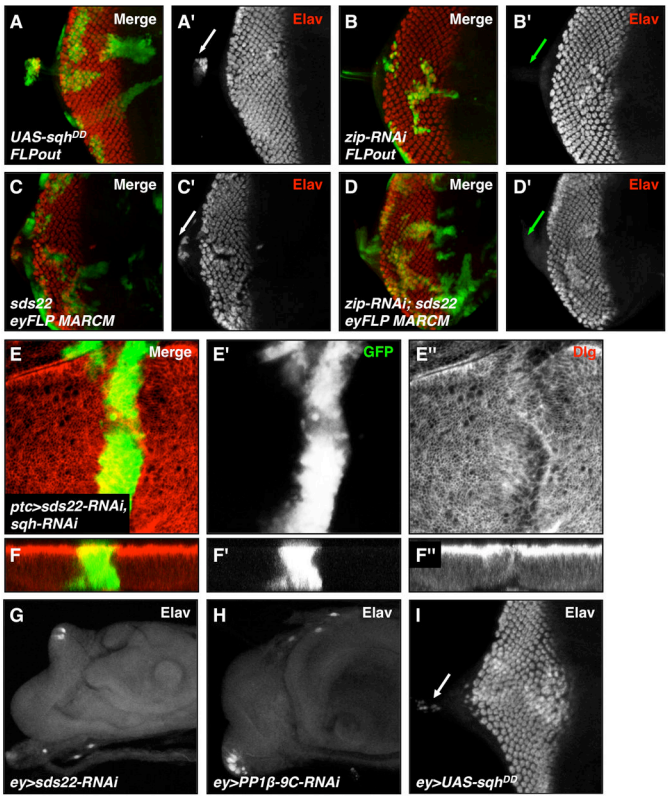

Figure 5. Nonmuscle myosin II activity is required for cell invasion and tissue integrity changes caused by loss of *sds22*

(A–D) Third instar eye discs stained with GFP (green, positively marks clones) and Elav (red, marks neurons) are shown. (A) Increasing myosin II activity by overexpression of a phosphomimetic form of Sqh (sqh^{DD}) using the *FLPout* method induces cell invasion into the optic stalk (A′, white arrow). (B) Reducing myosin II activity by knockdown of *zip* activity using the *FLPout* technique causes differentiation defects without inducing cell invasion (lack of Elav, red channel) into the optic stalk (B′, green arrow). (C) Loss of *sds22* using the *eyFLP/*MARCM method causes a cell invasion phenotype (C′, white arrow). (D) Coexpression of an RNAi construct against *zip* can suppress the cell invasion phenotype caused by loss of *sds22* (indicated by lack of Elav-positive cells in the optic stalk; D′, green arrow), suggesting that myosin II acts downstream of *sds22* to mediate cell invasion. (E–F) Reducing myosin II activity by knockdown of *sqh* activity using the *ptc-GAL4* driver suppresses both the cell invasion and apical abnormal folding phenotypes of *sds22* deficient cells. GFP (green) marks mutant cells and Dlg (red) marks the tissue morphology. (G–I) knockdown of either *sds22* (G) or *PP1β* (H) by*ey-GAL4* causes a dramatic loss of differentiation as indicated by a nearly complete loss of Elav staining. However, overexpression of *UAS-*sqh^{DD} (I) by *ey-GAL4* causes a less severe phenotype with Elav-positive cells mislocalized in the optic stalk (white arrow).

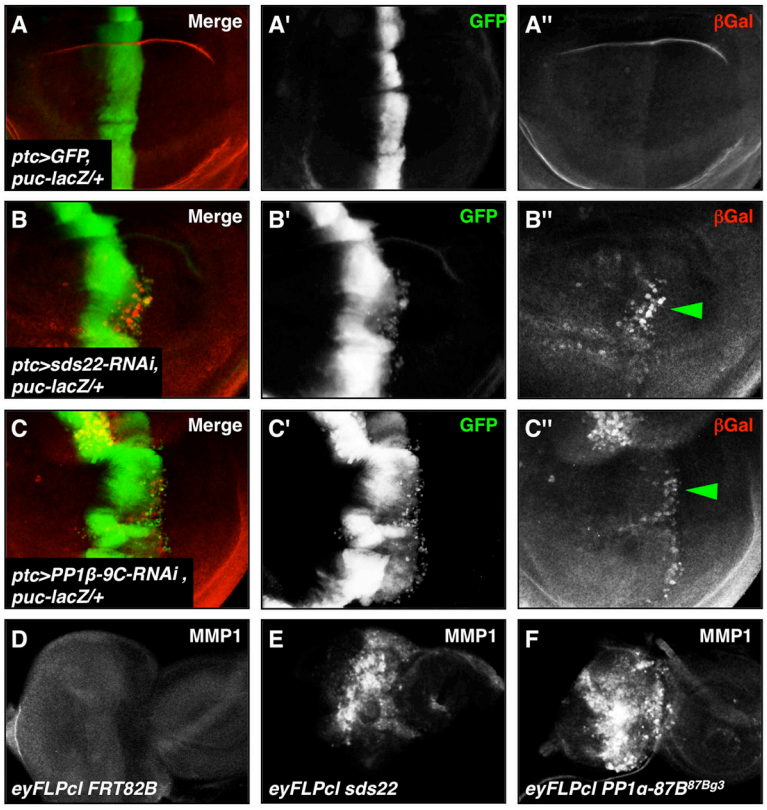

Figure 6. Loss of either *sds22* or *PP1* leads to increased JNK signaling and MMP1 expression

(A–C) *ptc-GAL4*-expressing cells are visualized by GFP (green) and *puc-lacZ* reporter expression is revealed by anti-βGal (red). Transcription of a *puc-lacZ* reporter gene is normally low or absent in the wing disc (A). Increased *puc-lacZ* expression in *sds22* or *PP1β* mutant migrating cells (B″,C″, green arrowheads) suggests that loss of either *sds22* or *PP1β* activates *puc* transcription in response to JNK pathway activation. (D–F) Third instar eye-antennal discs are labeled with MMP1, another downstream target of JNK signaling. Compared to the control disc (D), *sds22* (E) or *PP1α* (F) mutant discs show strong activation of MMP1 expression.

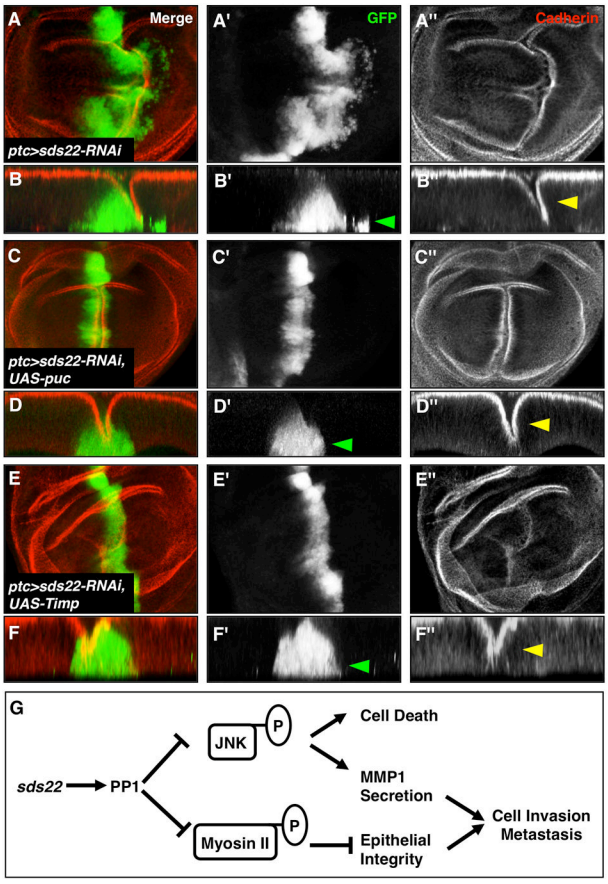

Figure 7. JNK activity is required for *sds22*-mediated cell invasion and overgrowth

(A–D) Blocking the JNK pathway by coexpression of *puc* (a feedback inhibitor of JNK) in *sds22* mutant cells suppresses the cell invasion phenotype caused by loss of *sds22* (loss of GFP-positive cells in the posterior compartment, D′ compared to B′, green arrowheads). Note that disruption of the cell adhesion marker Cadherin (red) is not suppressed by *puc* expression (D″ compared to B′, yellow arrowheads). (E,F) Overexpression of *Timp*, an inhibitor of MMPs, also largely suppresses the cell invasive behavior of *sds22* mutant cells (F′, green arrowhead). Tissue integrity indicated by Cadherin staining is not suppressed (F″, yellow arrowhead). (G) A model for *sds22* as a tumor suppressor gene in *Drosophila*. *sds22* functions as an essential positive regulator of PP1 to restrict two important signaling events that drive tumor metastasis: nonmuscle myosin II (myosin II) activity and the JNK signaling pathway. Hyperphosphorylation of myosin II leads to cytoskeleton reorganization and loss of epithelial integrity that is a criticaldeterminant of cell invasion and metastasis. Increased JNK signaling is required for *sds22*-mediated cell death and cell invasion likely through inducing MMP1 level.