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dGirdin a new player of Akt /PKB signaling in *Drosophila melanogaster*

Masamitsu Yamaguchi[1], Osamu Suyari[1], Rika Nagai[1], Masahide Takahashi[2]

[1]Department of Applied Biology, Insect Biomedical Research Center, Kyoto Institute of Technology, Sakyo-ku, Kyoto 606-8585, Japan, [2]Department of Pathology, Nagoya University Graduate School of Medicine, Showa-ku, Nagoya, Aichi 466-8550, Japan

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Girdin: its in vivo role as the Akt substrate
3.1. Identification of Girdin family proteins
3.2. Role of Akt in cell size control
3.3. Expression pattern of dGirdin PA and dGirdin PB
3.4. dGirdin is necessary for viability of Drosophila
3.5. dGirdin is required to control appropriate cell size in Drosophila wings
3.6. dGirdin is necessary for actin filament accumulation and may function downstream of the Akt
3.7. Role of Girdin in progression of human cancers
3.8. Drosophila models for tumor metastasis and angiogenesis
4. Summary and perspective
5. Acknowledgements
References

1. ABSTRACT

A novel substrate of Akt/PKB designated as Girdin (griders of actin filaments) has been identified in mammals and characterized as an actin-binding protein. A Girdin-like protein has been identified in *Drosophila*, which has two isoforms, dGirdin PA and dGirdin PB. Knockdown of dGirdin in the *Drosophila* wing imaginal disc cells resulted in reduction of cell size and this was enhanced by half reduction of the *Akt* gene dose. Furthermore the dGirdin-knockdown wing disc cells exhibited severe disruption of actin filaments. From these *in vivo* analyses, we conclude that dGirdin is required for actin organization and regulation of appropriate cell size under control of the Akt signaling pathway. Human Girdin plays important roles in cancer progression and angiogenesis. Therefore Girdin and its interacting proteins could be potential pharmaceutical targets for cancer therapies and tumor angiogenesis. Possible use of the *Drosophila* Girdin model in understanding the mechanisms of cancer progression and in developing preventive and therapeutic strategies will be discussed.

2. INTRODUCTION

Coordinate control of cell size, division and death plays important roles during development(1). In multicellular organisms, the control of cell size is necessary for dictating the proportions of organs, limb and the living entity(2). Cell size is regulated by a number of cellular signal molecules and one crucial example is the serine/threonine kinase Akt (also named Protein Kinase B, PKB). Akt is well known as the key regulator of many signal transduction pathways, modulating multiple processes including proliferation, growth, migration and cellular metabolism(3-6). Akt also negatively regulates apoptosis by phosphorylating and inactivating various components of the apoptotic machinery(7). Akt has a wide range of its substrates involved in the various cellular processes(8). A novel substrate of Akt designated as Girdin (griders of actin filaments) has been first identified in mammals(9). Recently we have identified a Girdin-like protein in *Drosophila* (dGirdin) (10). In this review, we summarize the roles of dGirdin in comparison with those of its mammalian counterparts, with a particular emphasis on

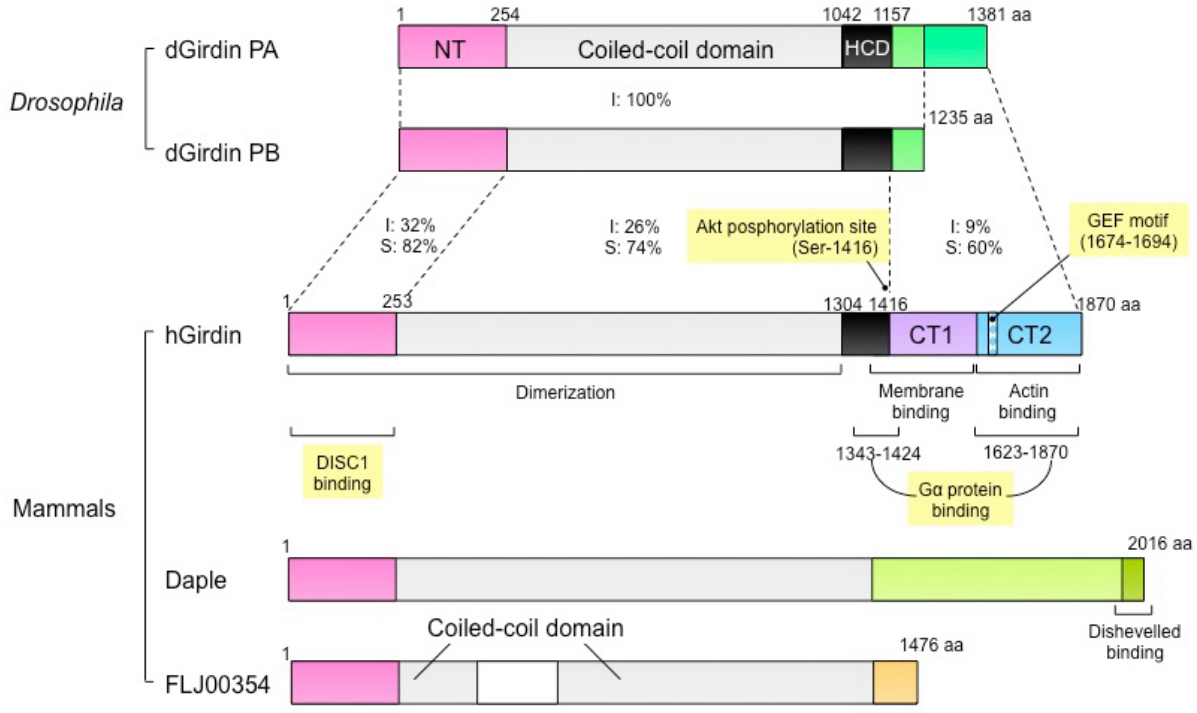

Figure 1. Structure and conserved domains of *Drosophila* and mammalian Girdin. *Drosophila* Girdin (dGirdin) gene has two alternative sites for translation termination that gives rise to two isoforms of the protein named dGirdin PA and dGirdin PB. The primary structures of human Girdin (hGirdin), Daple and FLJ00354, members of the Girdin protein family are also shown. N-terminal domain (NT) that shows high homology with the microtuble-binding domain of the Hook family of proteins, a central coiled-coil domain, highly conserved domain (HCD) and a C-terminal domain that binds to actin filaments and the Gα protein family are shown. The N-terminal and coiled coil domains contribute to Girdin oligomerization. I, identity; S, similarity.

its role on cell size control. We also focus on the emerging roles of human Girdin in cancer progression(11-13). A wealth of information on *Drosophila* development and genetics, and the existence of a number of mutants and transgenic lines, may significantly facilitate use of the *Drosophila* model in deciphering the mechanisms of cancer progression and in developing preventive and therapeutic strategies.

3. GIRDIN: ITS *IN VIVO* ROLES AS THE AKT SUBSTRATE

3. 1. Identification of Girdin family proteins

Mammalian Girdin has been identified as a novel protein that interacts with Akt(9, 14), the trimeric G protein Gαi/s(15) and dynamin(16), a large GTPase necessary for endocytosis(17). Human and mouse Girdin genes encode proteins of 1,870 and 1,845 amino acids, respectively (Figure 1). Girdin carries α-helical coiled-coil domain that spans more than two-thirds of the protein in its middle domain (the coiled-coil domain). The N-terminal domain (253 amino acids) shows significant homology to the microtubule-binding domain identified in the Hook family of proteins that link organelles to microtubules(16). Both of these domains contribute to the oligomerization of Girdin. Genome database analyses revealed Girdin paralogues, Daple (Dishevelled-associating protein with a high frequency of leucine residues) and FLJ00354, in the human and mouse genomes. It is known that Daple is a binding partner and regulator of Dishevelled, an important cytoplasmic component of the Wnt signaling pathway(18). *Drosophila* genome contains a single *Girdin*-like gene, CG12734 that encodes two proteins of 1,381 and 1,235 amino acids possibly produced by alternative splicing at the 3' region. These proteins were named dGirdin PA and dGirdin PB, respectively. The N-terminal and the coiled-coil domains between human and *Drosophila* Girdin show high homology, approximately 82% and 74 % similarity, respectively (Figure 1). Akt phosphorylates human Girdin at Ser-1417(9). The Akt phosphorylation site-like amino acid sequences are also conserved in dGirdin at similar amino acid position of human Girdin(10).

3. 2. Role of Akt in cell size control

Akt activity is principally determined by the level of phosphatidylinositol-3,4,5-triphosphate (PIP3) in the plasma membrane. Upon stimulation of receptor tyrosine kinase by growth factor stimulation, PIP3 is generated by the phosphatidylinositol-3-kinase (PI3K), which itself is counteracted by the lipid phosphatase, PTEN(19). When PIP3 levels are elevated, Akt is recruited to the plasma membrane and phosphorylated. Expression of Akt in *Drosophila* and mammalian tissues results in marked increase in cell growth/size and Akt signaling has been

shown to regulate cell growth via the Tsc/Rheb/TOR (target of rapamycin) pathway(19). Signaling from Akt disrupts the Tsc complex, activating Rheb and consequently activating TOR, which directly stimulates ribosomal protein p70 S6 kinase (S6K) and thus stimulates translation of mRNAs encoding ribosomal proteins(20, 21). In fact *Drosophila* deficient in the *S6 kinase* gene (*dS6K*) exhibited extreme delay in development and severe reduction in body size(2). Thus, the control of cell size by the PI3K/Akt pathway has been in part explained by its ability to regulate protein synthesis by TOR, ribosomal protein p70 S6 kinase, and eukaryotic initiation factor 4E-binding protein-1 (eIF4E-BP1)/ PHAS-I(22-24). However, cell growth or increase in cell size also depends on enlargement of the actin cytoskeleton architecture and the control of actin organization by Akt/PI3K pathway is still poorly understood.

3. 3. Expression pattern of dGirdin PA and dGirdin PB

Both dGirdin PA and dGirdin PB are expressed in various tissues including wing imaginal discs throughout all developmental stages of *Drosophila*(10). The levels of dGirdin PA are high in unfertilized eggs and embryos. Maximum levels were observed in 6- to 12-h embryos with subsequent remarkable decrease before entering the larval stage. dGirdin PA levels then decreased slightly across the adult stage(10). Enrichment of dGirdin PA mRNA in adult ovary and tubules are also described in the database (FlyAtlas, http://flyatlas.org/). In contrast, dGirdin PB levels decreased in the early stage of embryogenesis and were then slightly increased in 12- to 18-h embryos, thereafter decreasing in a manner similar to dGirdin PA(10). The high levels of dGirdin PA and dGirdin PB in unfertilized eggs indicate maternal expression. The results also suggest that dGirdin PA might play more important roles than dGirdin PB during embryogenesis.

Mammalian Girdin is also expressed in virtually all tissues and in various cell lines(13). However in mouse embryonic development, Girdin expression is temporally and spatially restricted (16). At embryonic day (E) 10.5, Girdin mRNA was detected in the branchial arches, nasal processes, limbs, somites and dorsal root ganglia. At E11.5-E12.5, its expression persists at these sites and was also detected in the fore-, mid-, and hindbrain, and the interdigital mesenchyme of the limbs as well as in the developing eye.

3. 4. dGirdin is necessary for viability of *Drosophila*

In order to investigate the function of dGirdin in living flies, transgenic flies carrying *UAS-dGirdin-IR* were crossed with *Actin5C-GAL4* driver lines to knockdown *dGirdin* in all fly tissues and the progeny flies exhibited late pupal lethality(10). Western blot analysis using anti-dGirdin antibodies revealed preferential knockdown of dGirdin PA by dGirdin-dsRNA in whole bodies, although it was designed to knockdown both mRNAs. Therefore it is suggested that at least dGirdin PA is necessary for viability of *Drosophila*. In accordance with this, the dGirdin mutants also show homozygous lethal phenotype, although the lethal phase is not precisely determined yet(10).

The Girdin deficient mice did not show apparent gross abnormalities at birth, but they displayed weight loss after postnatal day 7-8 (P7-8) and died by P25 without any serious pathological changes(12, 25). Therefore mouse Girdin is also necessary for viability of mouse. Detailed studies with Girdin-knockout mice revealed that Akt-mediated phosphorylation of Girdin promotes vascular endothelial growth factor (VEGF)-mediated postneonatal angiogenesis(12). Moreover Girdin-knockout mice show severe defects in the cytoarchitecture of the denate gyrus of the hippocampus that is known to develop postnatally(25). The defect is due to abnormal migration and/or positioning of newborn neurons in the dentate gyrus that is regulated by the interaction of Girdin with Disrupted-In-Schizophrenia 1 (DISC 1), a susceptibility gene for major mental illness including schizophrenia(26, 27). It is noted that the data suggest the interesting role of Girdin in pathoetiology of these psychiatric diseases.

3. 5. dGirdin is required to control appropriate cell size in *Drosophila* wings

The *Drosophila* wing is an appropriate tissue to investigate cell size control. Since each wing cell is known to produce one hair during development, one can easily measure cell density in the defined wing area by simply counting the hairs(28). Knockdown of dGirdin using an *en*-GAL4 driver line which drives expression of dGirdin-dsRNA in cells in the posterior compartment of the wing resulted in a reduction of posterior wing area (Figure 2) (10). Specific reduction of both dGirdin PA and PB in the posterior compartment of wing imaginal discs was confirmed by immunostaining with the anti-dGirdin antibody recognizing both dGirdin PA and PB(10). To examine whether the reduction in posterior wing area was caused by a decrease in cell size or a loss of cell number, we measured cell density in posterior wing areas in each fly by counting the hairs in defined areas, as each wing cell is known to produce one hair during development. The results revealed posterior wing compartments in which dGirdin was knocked down to exhibit higher cell density than controls and that this effect correlates with the copy number of the *UAS-dGirdin-IR* transgene. The data thus indicate that reduction in the posterior wing area induced by knockdown of dGirdin is due to decrease in cell size (10).

To exclude off-target effects of dGirdin-knockdown experiments, we examined whether the reduction in posterior wing area observed upon dGirdin-knockdown was sensitive to mutation in the *dGirdin* gene. On the *dGirdin* mutant background, reduction of posterior region of the dGirdin knockdown wings was further enhanced and cell density increased(10). The results indicate that the wing phenotype induced by dGirdin knockdown is indeed due to reduction of dGirdin.

3. 6. dGirdin is necessary for actin filament accumulation and may function downstream of the Akt

Human Girdin has been identified as a substrate of Akt functioning under the control of Akt phosphorylation(9). To determine whether dGirdin functions in a similar manner, we examined genetic

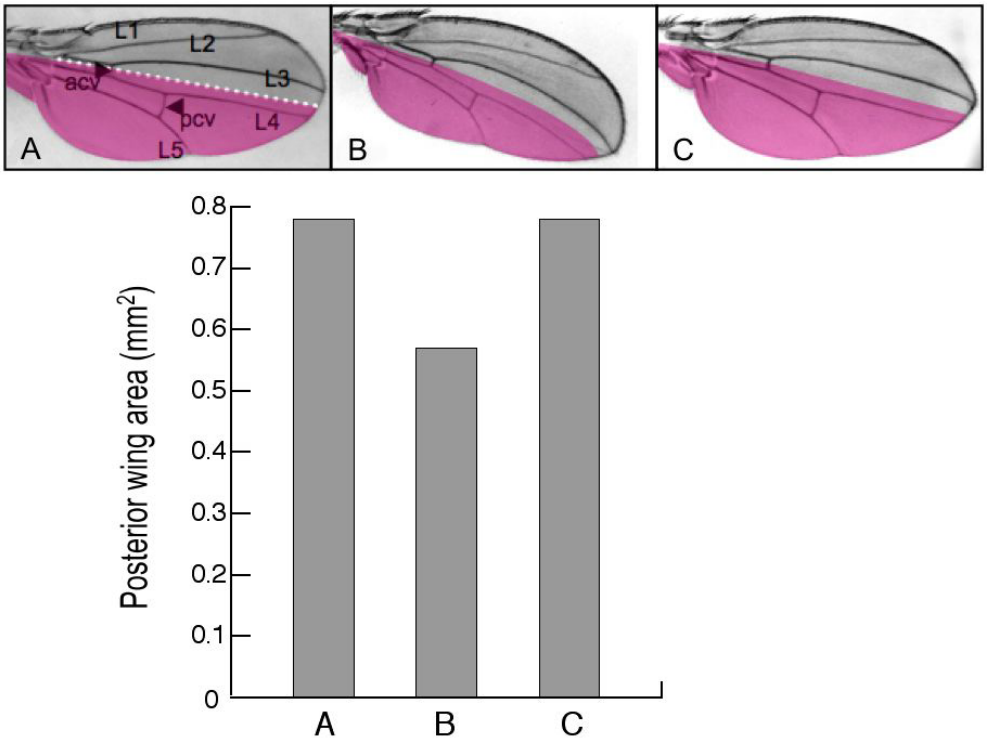

Figure 2. Microscopic images of adult wings. (A) *en-GAL4*/+; + (control), (B) *en-GAL4*/+; UAS-dGirdin-IR (dGirdin-knockdown), (C) UAS-Akt/+; en-GAL4/+; UAS-dGirdin-IR/+ (rescue by Akt-overexpression). The broken line in (A) indicates the anterior-posterior boundary. The red areas indicate posterior wing compartments. L, acv and pcv indicate longitude wing vein, anterior cross vein and posterior cross vein, respectively. The area of posterior wing compartment was reduced in dGirdin-knockdown fly (B) and this was rescued by Akt-overexpression (C).

interactions between *dGirdin* and *Akt* in *Drosophila* by observing the effects of *Akt* mutation on wing phenotype induced by knockdown of dGirdin(10). Half reduction of the *Akt* gene dose strongly enhanced the wing phenotype, with dramatic reduction in cell size in the wing. To further confirm *in vivo* roles of dGirdin under Akt influence, we examined the effect of Akt-overexpression on the wing phenotype (10). Overexpression of Akt in *Drosophila* wing using the *en*-GAL4 driver enlarged the posterior wing compartment area. In addition, the cell density was rather decreased, consistent with the widely known function of Akt in upregulating cell growth in a cell-autonomous manner. However, when dGirdin was knocked down in posterior compartments in which Akt was overexpressed, the elevation of cell size was suppressed with rescue to the normal level (Figure 2). These observations, taken together, suggest that dGirdin functions downstream of Akt in the control of cell size of the wing (Figure 3).

Mammalian Girdin is reported to be an actin-binding protein, which functions under the control of Akt(9). In human fibroblast cells, Girdin was shown to be essential for organization of the actin cytoskeleton, actin stress fibers being disrupted in Girdin-knockdown cells with loss of shape and formation of rugged boundaries(9). In wing imaginal discs from flies in which dGirdin was specifically knocked down in the posterior region by using *en*-GAL4 driver, immunofluorescent staining with polyclonal anti-dGirdin antibody showed that the level of dGirdin was considerably reduced in the posterior region. When actin filaments were visualized by staining with phalloidin, the region in which dGirdin was knocked down exhibited low accumulation(10). These results indicate that dGirdin is essential for the organization of the actin filaments in *Drosophila* as with its human counterpart (Figure 3).

3. 7. Role of Girdin in progression of human cancers

It is well known that aberrant Akt signaling is involved in the initiation and progression of human cancers (29-34). Therefore it is likely that the Akt/Girdin pathway also contributes to the malignant behavior of cancer cells. Many case studies revealed that Girdin is expressed in a wide variety of cancer cell lines and tissues including epithelium-derived and endothelium-derived malignant tumors, suggesting that Girdin plays important roles in cancer development and/or progression(11, 12, 35, 36).

Many cancer cells possess the ability to invade and metastasize to adjacent and distant tissues. The cytoskeleton is the force-generating apparatus that moves cells from one place to another and actin filaments regulate many aspects of dynamic cell motility(37). A number of actin-binding proteins and Rho GTPases, which are controlled by a variety of intercellular signaling such as the PI3K/Akt pathway, play crucial roles in regulating actin

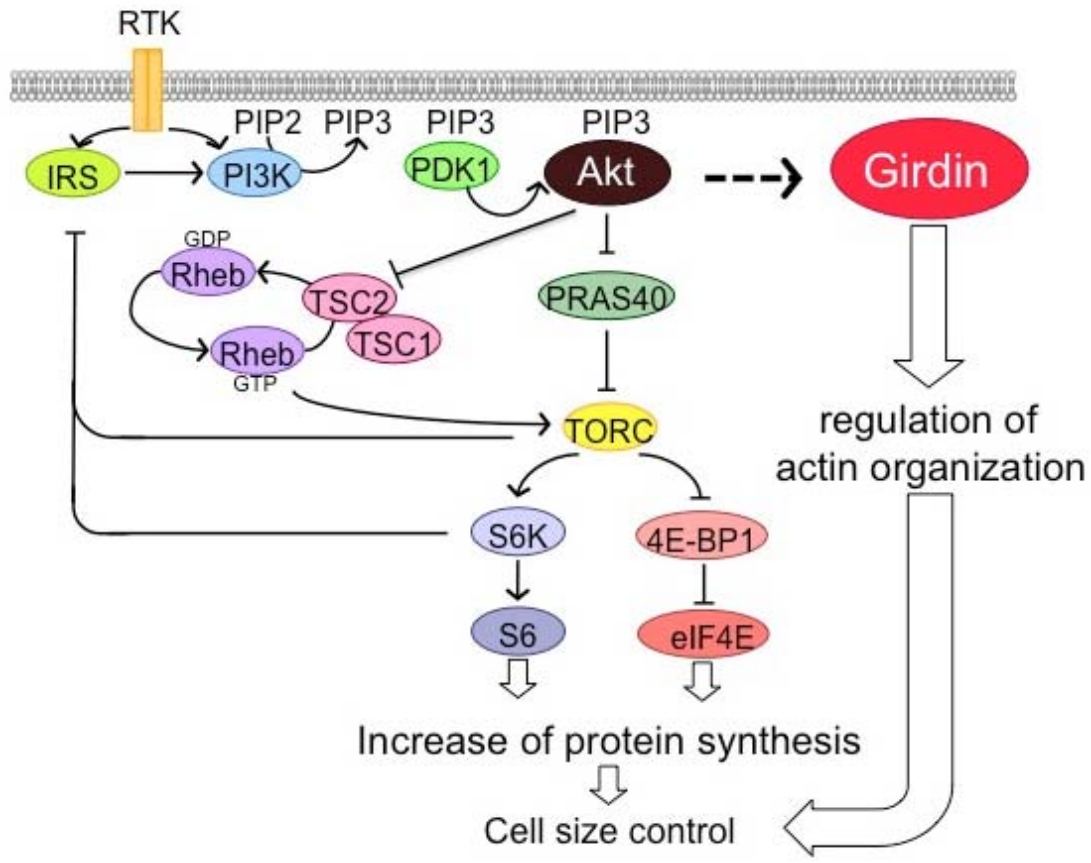

Figure 3. Role of Girdin in actin organization and cell size control. The binding of various growth factors to cell-surface receptors triggers an activation of Akt through PI3K. The activated Akt subsequently phosphorylates several substrates including Girdin to regulate actin organization and cell size control.

reorganization(38, 39). Many lines of evidence indicates that the PI3K/Akt signaling pathway also regulates the migration of cancer cells through several mechanisms such as phosphorylation of Girdin at the leading edge of migrating cells(11). In quiescent cells without growth factors, Girdin primarily localizes along actin stress fibers(15). However in migrating cells, Girdin preferentially localizes to the actin meshwork structure of the lamellipodia at the leading edge(9, 11, 12). In response to migratory cues such as growth factors, the phosphorylation of Girdin by Akt occurs at the leading edge, which is required for directional cell migration that in case of cancer cells ultimately leads to invasion and metastasis(13).

Angiogenesis is necessary for tumorigenesis including metastasis and therefore inhibiting angiogenesis has advantages as a cancer treatment strategy (40, 41). Among various factors involved in angiogenesis, members of the vascular endothelial growth factor (VEGF) and angiopoietin families have predominant roles(40, 42). Girdin is highly expressed in human umbilical vein endothelial cells and its phosphorylation by Akt regulates VEGF/Akt-dependent angiogenesis *in vitro* and *in vivo*(12). Phenotypic analysis of Girdin-knockout mice showed that Girdin is dispensable for embryonic vascular development but is required for postnatal vascular formation(12). Thus Girdin, although vital for cancer progression and postnatal vascular remodeling, is dispensable for cell migratory events during embryonic development. These findings suggest that Girdin and its interacting proteins are potential pharmaceutical targets for cancer therapies and tumor angiogenesis.

3. 7. *Drosophila* models for tumor metastasis and angiogenesis

Mutations in several tumor suppressor genes in *Drosophila*, including *lethal(2)giant larvae* (*lgl*)*, discs large* (*dlg*) and *scribble* (*scrib*), are well known to promote tissue overgrowth(43-45). These proteins are widely expressed and normally act together to regulate apical-basal polarity in epithelial cells. Although the mechanism by which these proteins control polarity is not entirely clear at the molecular level, they are proposed to work as scaffolding proteins that control the segregation of membrane proteins to distinct domains of the cell (46). In addition, they affect downstream signaling events such as the activation of Rho GTPases. By taking advantage of mutations in these tumor suppressor genes, two different systems were developed to generate marked tumors *in vivo*

and then follow tumor cells that have moved to other places (46).

In a first system, tumor cells expressing a reporter gene such as *lacZ* are dissected from *lgl* or *dlg* mutant larvae and then transplanted into the abdomens of adult hosts(44). Later the marked cells can be identified even in distant places to the site of injection by simply immunostaining with anti-lacZ antibody (46). Since invasive cells are able to cross multiple cell layers and basement membranes during their spread, this *Drosophila* model could be used to genetically identify other genes required for these events (47). A second system took advantage of the sophisticated genetic tricks in *Drosophila* to generate GFP-marked mutant clones expressing either a gain-of-function allele of *Ras*, Ras^{V12}, or homozygous for loss-of-function mutations in *scrib* while simultaneously altering additional genetic pathways (48, 49). The mutations in *scrib*, in the Ras^{V12} background results in tumor cells to invade neighboring cells. It would be interesting to examine role of dGirdin in these metastasis systems in *Drosophila*.

Drosophila developed a tube-like heart and an open circulatory system for blood cells and there are no blood or lymphatic vessels. However, the development of the tracheal system in *Drosophila* very likely represents a model for angiogenesis in human (50, 51). In *Drosophila* less than 100 cells in each half-segment organize themselves into tubes. Cells at the tips of the tubes grow out into peripheral tissues to form fine branches as they ramify along stereotyped trajectories. Branchless, a FGF-like protein plays a major role in patterning the branches and guiding their outgrowth. Branchless binds and activates a receptor tyrosine kinase Breathless (51, 52). It should be noted that dGirdin is highly expressed in the tracheal system and may play a role in development of the tracheal system in *Drosophila*.

4. SUMMARY AND PERSPECTIVES

A novel Girdin-like protein has been identified in *Drosophila*, which has two isoforms, dGirdin PA and dGirdin PB. Knockdown of dGirdin in the *Drosophila* wing imaginal disc cells resulted in reduction of cell size. In the wing disc, dGirdin was also required for accumulation of actin filaments. Furthermore, these effects were enhanced by half reduction of the *Akt* gene dose. From these *in vivo* analyses, we conclude that dGirdin is required for actin organization and regulation of appropriate cell size under control of the Akt signaling pathway (Figure 3). Many lines of evidence indicate that mammalian Girdin plays important roles in cancer progression and angiogenesis(13). An important observation is that Girdin is dispensable for cell migratory events during mouse embryonic development, although it is required for cancer progression and postnatal vascular remodeling. These findings suggest that Girdin and its interacting proteins are potential pharmaceutical targets for cancer therapies.

Drosophila is becoming a powerful model where many aspects of the tumorigenesis can be genetically recapitulated (53). In general *Drosophila* has the practical advantages of being small, highly fertile and having a short generation time. These characteristics allow large-scale genetic screens and high-throughput screen of candidate drugs for therapy, to be performed in a relatively short period of time and at low cost (54, 55). dGirdin-knockdown and dGirdin-overexpressing flies showed a characteristic wing phenotype. These transgenic flies should prove to be useful tools for genetically identifying novel targets of dGirdin and its positive or negative regulators in *Drosophila*. Human counterparts of these genetic interactants with the *dGirdin* gene could be potential pharmaceutical targets for cancer therapies and tumor angiogenesis in which human Girdin is involved. In addition these transgenic flies would be useful to screen various chemicals and natural substances that could modify the dGirdin activity. This screen could be a first step to identify candidate drugs for therapy of human diseases including cancer in which human Girdin is involved.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

1. R. S. S. Fraser, and P. Nurse. Novel cell cycle control of RNA synthesis in yeasts. *Nature* 27 726-730 (1978)

2. J. Montagne, M. J. Stewart, H. Stocker, E. Hafen, S. C. Kozma, and G. Thomas. *Drosophila* S6 kinase: a regulator of cell size. *Science* 285 2126-2129 (1999)

3. D. P. Brazil, J. Park, and B. A. Hemmings. PKB binding proteins: Getting in on the Akt. *Cell* 111 293-303 (2002)

4. S. R. Datta, A. Brunet, and M. E. Greenberg. Cellular survival: a play in three Akts. *Genes Dev*. 13 2905-2927 (1999)

5. I. Vivanco, and C. L. Sawyers. The phosphatidylinositol 3-kinase-Akt pathway in human cancer. *Nat. Rev. Cancer* 2 489-501 (2002)

6. S. Merlot, and R. A. Firtel. Leading the way: directional sensing through phosphatidylinositol 3-kinase and other signaling pathway. *J. Cell Sci*. 116 3471-3478 (2003)

7. S. R. Datta, H. Dudek, X. Tao, S. Masters, H. Fu, Y. Gotoh, and M. E. Greenberg. Akt phospholylation of Bad couples survival signals to cell-intrinsic death machinery. *Cell* 91 231-241 (1997)

8. B. D. Manning, and L. C. Cantley. AKT/PKB signaling:navigating downstream. *Cell* 129 1261-1274 (2007)

9. A. Enomoto, H. Murakami, N. Asai, N. Morone, T. Watanabe, K. Kawai, Y. Murakumo, J. Usukura, K. Kaibuchi, and M. Takahashi. Akt/PKB regulates actin

organization and cell motility via Girdin/APE. *Dev. Cell* 9 389-402 (2005)

10. A. Puseenam, Y. Yoshioka, R. Nagai, R. Hashimoto, O. Suyari, M. Itoh, A. Enomoto, M. Takahashi, and M. Yamaguchi. A novel *Drosophila* Girdin-like protein is involved in Akt pathway control of cell size. *Exp. Cell Res*. 315 3370-3380 (2009)

11. P. Jiang, A. Enomoto, M. Jijiwa, T. Kato, T. Hasegawa, M. Ishida, S. T., N. Asai, Y. Murakumo, and M. Takahashi. An actin-binding protein Girdin regulates the motility of breast cancer cells. *Cancer Res*. 68 1310-1318 (2008)

12. T. Kitamura, N. Asai, A. Enomoto, K. Maeda, T. Kato, M. Ishida, P. Jiang, T. Watanabe, J. Usukura, T. Kondo, F. Constantini, T. Murohara, and M. Takahashi. Regulation of VEGF-mediated angiogenesis by the Akt/PKB substrate Girdin. *Nature Cell Biol*. 10 329-337 (2008)

13. L. Weng, A. Enomoto, M. Ishida-Takagishi, N. Asai, and M. Takahashi. Girdin for migrating cues: roles of the Akt substrate Girdin in cancer progression and angiogenesis. *Cancer Sci*. in press (2010)

14. M. Anai, N. Shojima, H. Katagiri, T. Ogihara, H. Sakoda, Y. Onishi, H. Ono, M. Fujishiro, Y. Fukushima, and N. Horike. A novel protein kinase B (PKB)/AKT-binding protein enhances PKB kinase activity and regulates DNA synthesis. *J. Biol. Chem*. 280 18525-18535 (2005)

15. H. Le-Niculescu, I. Niesman, T. Fischer, L. DeVries, and M. G. Farquhar. Identification and characterization of GIV, a novel Gα i/s-interacting protein found on COPI, endoplasmic reticulum-Golgi transport vesicles. *J. Biol. Chem*. 280 22012-22020 (2005)

16. F. Simpson, S. Martin, T. M. Evans, M. Kerr, D. E. James, R. G. Parton, R. D. Teasdale, and C. Wicking. A novel Hook-related protein family and the characterization of Hook-related protein 1. *Traffic* 6 442-458 (2005)

17. G. J. Praefcke, and H. T. McMahon. The dynamin superfamily:universal membrane tubulation and fission molecules? *Nat. Rev. Mol. Cell Biol.* 5 133-147 (2004)

18. A. Oshita, S. Kishida, H. Kobayashi, T. Michiue, T. Asahara, M. Asashima, and A. Kikuchi. Identification and characterization of a novel Dvl-binding protein that suppresses Wnt signalling pathway. *Genes Cells* 8 1005-1017 (2003)

19. N. Hay. The Akt-mTOR tango and its relevance to cancer. Cancer Cell 8 179-183 (2005)

20. C. J. Potter, L. G. Pedraza, and T. Xu. Akt regulates growth by directly phosphorylating Tsc2. *Nat. Cell. Biol.* 4 658-665 (2002)

21. K. M. Hennig, J. Colombani, and T. P. Neufeld. TOR coordinates bulk and targeted endocytosis in the *Drosophila melanogaster* fat body to regulate cell growth. *J. Cell Biol.* 173 963-974 (2006)

22. A. L. Edinger, and C. B. Thompson. Akt maintains cell size and survival by increasing mTOR-dependent nutrient uptake. *Mol. Biol. Cell* 13 2276-2288 (2002)

23. D. C. Fingar, S. Salama, C. Tsou, E. Harlow, and J. Blenis. Mammalian cell size is controlled by mTOR and its downstream targets S6K1 and 4EBP1/eIF4E. *Genes Dev*. 16 1472-1487 (2002)

24. T. Radimerski, J. Montagne, F. Rintelen, H. Stocker, J. van der Kaay, C. P. Downes, E. Hafen, and G. Thomas. dS6K-regulated cell growth is dPKB/dPI(3)K-independent, but requires dPDK1. *Nat. Cell Biol.* 4 251-255 (2002)

25. A. Enomoto, N. Asai, T. Namba, Y. Wang, T. Kato, M. Tanaka, H. Tatsumi, S. Taya, D. Tsuboi, K. Kuroda, N. Kaneko, K. Sawamoto, R. Miyamoto, M. Jijiwa, Y. Murakumo, M. Sokabe, T. Seki, K. Kaibuchi, and M. Takahishi. Roles of disrupted-in-schizophrenia 1-interacting protein girdin in postnatal development of the dentate gyrus. *Neuron* 63 774-787 (2009)

26. J. K. Millar, J. C. Wilson-Annan, S. Anderson, S. Christie, M. S. Tayler, C. A. Semple, R. S. Devon, D. M. St. Clair, W. J. Muir, D. H. Blackwood, and D. J. Porteous. Disruption of two movel genes by a translocation co-segregating with schizophrenia. *Hum. Mol. Genet.* 9 1415-1423 (2000)

27. J. Y. Kim, X. Duan, C. Y. Liu, M. H. Jang, J. U. Guo, N. Pow-anpongkul, E. Kang, H. Song, and G. L. Ming. DISC1 regulates new neuron development. *Neuron* 63 711-713 (2009)

28. C. A. Meyer, H. W. Jacobs, S. A. Datar, W. Du, B. A. Edgar, and C. F. Lehner. *Drosophila* Cdk4 is required for normal growth and is dispensable for cell cycle progression. *EMBO. J*. 19 4533-4542 (2000)

29. J. Luo, B. D. Manning, and L. C. Cantley. Targeting the PI3K-Akt pathway in human cancer: Rational and promise. *Cancer Cell* 4 257-262 (2003)

30. H. G. Wendel, E. De Stanchina, J. S. Fridman, A. Malina, S. Ray, S. Kogan, C. Cordon-Cardo, J. Pelletier, and S. W. Lowe. Survival signalling by Akt and eIF4E in oncogenesis and cancer therapy. *Nature* 428 267-269 (2004)

31. Y. Samuels, and K. Erickson. Oncogenic PI3K and its role in cancer. *Curr. Opin. Oncol.* 18 77-82 (2006)

32. B. T. Hennessy, D. L. Smith, P. T. Ram, Y. Lu, and G. B. Mills. Exploiting the PI3K/AKT pathway for cancer drug discovery. *Nat. Rev. Drug Discov.* 4 988-1004 (2005)

33. E. Hernando, E. Charytonowicz, M. E. Dudas, S. Menendez, I. Matushansky, J. Mills, N. D. Socci, N. Behrendt, L. Ma, R. G. Maki, P. P. Pandolfi, and C. Cordon-Cardo. The AKT-mTOR pathway plays a critical role in the development of leiomyosarcomas. *Nat. Med.* 13 748-753 (2007)

34. J. Brognard, A. S. Clark, Y. Ni, and P. K. Dennis. Akt/protein kinase B is constitutively active in non-small cell lung cancer cells and promotes cellular survival and resistance to chemotherapy and radiation. *Cancer Res.* 61 3986-3997 (2001)

35. P. Ghosh, M. Garcia-Marcos, S. J. Bornheimer, and M. G. Farquhar. Activation of Gai3 triggers cell migration via regulation of GIV. *J. Cell Biol.* 182 381-393 (2008)

36. B. Zhang, F. Gu, C. She, H. Guo, W. Li, R. Niu, L. Fu, N. Zhang, and Y. Ma. Reduction of Akt2 inhibits migration and invasion of glioma cells. *Int. J. Cancer* 125 585-595 (2009)

37. T. B. Pollard, and G. G. Borisy. Cellular motility driven by assembly and disassembly of actin filaments. *Cell* 112 453-465 (2003)

38. A. J. Ridley, M. A. Schwartz, K. Burridge, R. A. Firtel, M. H. Ginsberg, G. Borisy, J. T. Parsons, and A. R. Horwitz. Cell migration: Integrating signals from front to back. *Science* 302 1704-1709 (2003)

39. S. Etienne-Manneville, and A. Hall. Rho GTPases in cell biology. *Nature* 420 629-635 (2002)

40. P. Carmeliet, and R. K. Jain. Angiogenesis in cancer and other diseases. *Nature* 407 249-257 (2000)

41. N. Ferrara, and R. S. Kerbel. Angiogenesis as a therapeutic target. *Nature* 438 967-974 (2005)

42. L. Coultas, K. Chawengsaksophak, and J. Rossant. Endothelial cells and VEGF in vascular development. *Nature* 438 937-945 (2005)

43. D. Bilder. Epithelial polarity and proliferation control: links from the *Drosophila* neoplastic tumor suppressors. *Genes Dev.* 28 1909-1925 (2004)

44. F. Froldi, M. Ziosi, G. Tomba, F. Parisi, F. Garoia, A. Pession, and D. Grifoni. *Drosophila lethal giant larvae* neoplastic mutant as a genetic tool for cancer modeling. *Current Genomics* 9 147-154 (2008)

45. P. O. Humbert, L. E. Dow, and S. M. Russell. The Scribble and Par complexes in polarity and migration: friends or foes? *Trends Cell Biol*. 16 622-630 (2006)

46. A. C.-C. Jang, M. Starz-Gaiano, and D. J. Montell. Modeling migration and metastasis in *Drosophila*. *J. Mammary Gland Biol. Neoplasia* 12 103-114 (2007)

47. E. C. Woodhouse, A. Fisher, R. W. Bandle, B. Bryant-Greenwood, L. Charboneau, E. F. Petricoin, and L. A. Liotta. *Drosophila* screening model for metastasis: Semaphorin 5c is required for *l(2)gl* cancer phenotype. *Proc. Natl. Acad. Sci. USA* 100 11463-11468 (2003)

48. A. M. Brumby, and H. E. Richardson. Using *Drosophila melanogaster* to map human cancer pathways. *Nat. Rev. Cancer* 5 626-639 (2005)

49. R. A. Pagliarini, and T. Xu. A genetic screen in *Drosophila* for metastasis behavior. *Science* 302 1227-1231 (2003)

50. R. J. Metzger, and M. A. Krasnow. Genetic control of branching morphogenesis. *Science* 284 1635-1639 (1999)

51. B. E. Kerman, A. M. Cheshire, and D. J. Andrew. From fate to function: the *Drosophila* trachea and salivary gland as models for tubulogenesis. *Differentiation* 74 326-348 (2006)

52. M. Affolter, S. Bellusci, N. Itoh, B. Shilo, J. P. Thiery, and Z. Werb. Tube or not tube: remodeling epithelial tissues by branching morphogenesis. *Dev. Cell* 4 11-18 (2003)

53. K. J. Venken, and H. J. Bellen. Emerging technologies for gene manipulation in *Drosophila melanogaster*. *Nat. Rev. Genet*. 6 167-178 (2005)

54. M. Leyssen, and B. Hassan. A fruitfly's guide to keeping the brain wired. *EMBO reports* 8 46-50 (2007)

55. E. Bier. *Drosophila*, the golden bug, emerges as a tool for human genetics. *Nat. Rev. Genet.* 6 9-23 (2005)

Abbreviations: Girdin, griders of actin filaments; PKB, Protein Kinase B; Daple, Dishevelled-associating protein with a high frequency of leucine residues; PIP3, phosphatidylinositol-3,4,5-triphosphate; PI3K , phosphatidylinositol-3-kinase; TOR, targeting rapamycin; VEGF, vascular endothelial growth factor; *lgl*, *lethal(2)giant larvae*; *dlg, discs large*; *scrib, scribble*

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Send correspondence to: Masamitsu Yamaguchi, Address: Department of Applied Biology, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan, Tel: 81-75-724-7781, Fax: 81-75-724-7799, E-mail: myamaguc@kit.ac.jp

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