

Review

Control of cell shape and plasticity during development and disease by the actin-binding protein Drebrin

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Summary. Drebrin is an actin-binding protein, originally identified in neuronal cells, involved in the regulation of actin filament organisation, especially during the formation of neurites and cell protrusions of motile cells. Drebrin is found in diverse non-neuronal cells, primarily in association with cell processes and intercellular junctions where it again plays a key role in actin remodelling. The downregulation of Drebrin in Alzheimer's Disease and Down Syndrome and conversely its upregulation in various carcinomas indicate that Drebrin is an important component of the pathogenesis of multiple diseases.

Key words: Drebrin, Actin, Alzheimer's Disease, Down Syndrome, Carcinoma

Introduction

The formation and maintenance of an appropriate shape is fundamental to the correct functioning of cells. Equally important is the ability to modulate morphology in response to changing environmental stimuli and pressures. In many instances, a change of shape must be accompanied by a change of location within the body, most notably during embryogenesis. Motile responses require not just regulated modifications of morphology but also communication with surrounding cells, particularly when a group must migrate collectively (Lecuit and Lenne, 2007; Friedl and Gilmour, 2009). The cytoskeleton is central to all these functions, providing both a rigid scaffold and forces to mould and

move the cell as appropriate. It is regulated by a host of proteins which bind to cytoskeletal components such as microtubules and actin filaments to modulate their turnover and dynamics. Drebrin is an actin-binding protein with a rapidly expanding repertoire of interactions with other proteins that implicates it in the control of cell shape and function throughout the body. There is increasing evidence that when molecular pathways linked to Drebrin go awry, the result is developmental abnormalities and disease.

Drebrin isoforms and functional domains

Drebrin, named from *developmentally regulated brain protein*, was first isolated from brains of 10-day chick (*Gallus gallus*) embryos (Kojima et al., 1988; Shirao et al., 1988). Three isoforms, E1 and E2 (embryonic) and A (adult), were found to be generated by alternative RNA splicing from a single Drebrin gene (Kojima et al., 1993). The isoforms differ by virtue of short inserted fragments: a 43 amino acid stretch (insert 1) is present in E2 but not E1 and a second insert of 46 amino acids (insert 2) is additionally present in A (Fig. 1). Thus far, only one embryonic isoform, originally named Drebrin E (Toda et al., 1993), has been detected in mammalian brains; this is more correctly called E2 as it contains insert 1 and is a homologue of chick E2. A C-terminally truncated form of Drebrin A, called s-Drebrin A, has been identified in postnatal murine brain (Jin et al., 2002) bringing the total number of identified Drebrin isoforms to four. All isoforms are strongly expressed in neurons; the expression pattern of each isoform is regulated spatially and temporally in the developing brain. Due to the acidic nature of Drebrin and post-translational modifications, its molecular weight, as judged by SDS-PAGE, is higher than that calculated from the amino acid sequence. The Drebrin protein is

highly conserved, especially in the amino-terminal (residues 1-315) which contains an actin-depolymerising factor homology (ADF-H) domain and an actin-binding domain (Fig. 1).

By various biochemical and immunocytochemical methods, all the Drebrin isoforms have been confirmed to bind filamentous actin (F-actin) through a high affinity actin-binding domain distinct from the ADF-H domain (Fig. 1). Rat Drebrin E2 binds actin filaments at a stoichiometry of 1:5, with a dissociation constant (K_d) of 1.2×10^{-7} M. Drebrin does not exhibit any actin nucleating, severing, capping or filament crosslinking activity (Shirao et al., 1994). It has been reported that Drebrin does not affect the activity of gelsolin, filamin, or caldesmon but inhibits the activity of other actin-binding proteins (Fig. 2), such as α -actinin, fascin, tropomyosin and myosinII (Ishikawa et al., 1994; Sasaki et al., 1996; Cheng et al., 2000). Overexpression of full length Drebrin, or truncations containing the actin-binding domain, induces the formation of numerous microspikes in fibroblasts or massive, abnormal spines in cultured hippocampal neurons (Hayashi et al., 1999; Mizui et al., 2005). The ADF-H domain present at the N-terminal has been suggested to compete with cofilin for binding to F-actin thereby contributing to the pathology of Alzheimer's Disease. This could be regulated by the binding site for 3-phosphoinositide-dependent protein kinase-1 (PDK1) located within the ADF-H domain (Chew et al., 2005). The actin-binding domain has also been suggested to have two actin binding sites, one of which is tropomyosin-sensitive and the other tropomyosin-insensitive (Ishikawa et al., 1994). Domain analysis indicates that the actin-binding domain contains two putative subdomains, one coiled-coil and one helical region. Both domains can induce the formation of large, spiky protrusions when overexpressed in fibroblast cells (our unpublished data); it remains to be established whether these differ in their sensitivity to tropomyosin. Drebrin also interacts with profilin, in this case through its proline-rich region (Mammoto et al., 1998). Profilin delivers monomeric, globular G-actin to the barbed end of actin filaments and accelerates actin polymerisation; this is one way therefore that Drebrin may influence actin dynamics. Chick Drebrin contains one (PPATF) and mammalian Drebrin contains two (PPATF and PPPVF) Homer binding sites in the C-terminal region (Fig. 1). Homer proteins are scaffolding molecules that facilitate the clustering of specific postsynaptic proteins, such as G protein-coupled glutamate receptors (mGluR), Shank

and the small GTPase Cdc42, thereby modulating their activities at neuronal synapses, believed to be important in learning and memory. Overexpression of Homer induces changes in dendritic spine morphology in cultured hippocampal neurons (Fagni et al., 2002; Shiraishi-Yamaguchi et al., 2009). Drebrin can interact with the N-terminal EVH-1 domain of Homer2 through its Homer binding motif. Moreover, Homer2 can interact with Cdc42 via its C-terminal thereby forming a Drebrin-Homer2-Cdc42 complex; this has been suggested to regulate spine morphology and synaptic properties (Shiraishi-Yamaguchi et al., 2009). Besides EVH-1, Drebrin also contains consensus binding sites for SH3, WW and PDZ domains (Chew et al., 2005); these have yet to be validated empirically. Recent data point to a wider role for Drebrin as a link between actin and another fundamental part of the cytoskeleton, the microtubule network. Drebrin specifically binds to EB3, a microtubule 'plus-end' protein, which associates with the tips of growing microtubules in neuronal growth cones. As extending microtubules reach the base of filopodia the EB3 on the tips interacts with Drebrin at the base of filopodia (Geraldo et al., 2008). This provides a zipper mechanism to guide the microtubule into the filopodium, thereby aligning and stabilising the actin and microtubule networks. Drebrin may thus act as a key part of the cytoskeletal machinery co-ordinating neuriteogenesis and growth cone dynamics. Indeed, suppression of Drebrin expression by antisense transfection attenuates neurite outgrowth in neuroblastoma B104 cells (Toda et al., 1999).

Drebrin was identified as a binding partner of the Connexin-43 (Cx43) carboxy-terminal domain (Fig. 2) in mouse brain homogenate (Butkevich et al., 2004). Cx43 is the most widely expressed member of the connexin family of proteins which form gap junctions between contacting cells. These mediate intercellular communication and signalling by permitting the passage of ions, metabolites and second messengers. Drebrin may therefore co-ordinate the linkage of Cx43 to the cytoskeleton by forming a bridge to microtubule-associated proteins and other juxtamembrane proteins, such as spectrin, to regulate the positioning and function of gap junctions (Butkevich et al., 2004; Geraldo et al., 2008). Deletion of the C-terminal domain of Cx43 alters neuronal migration in the neocortex (Cina et al., 2009), which further suggests that Drebrin may be involved in neuronal migration, reinforcing its importance for correct neural development. The Drebrin C-terminal has also been reported to interact with Ras to regulate

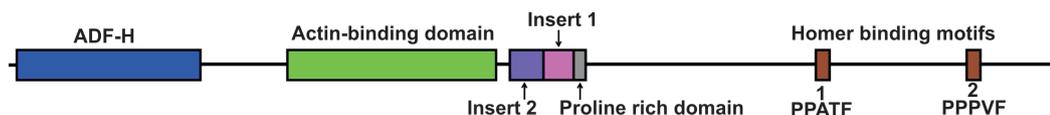


Fig. 1. Schematic diagram of Drebrin functional domains. The N terminal is highly conserved across vertebrate species and includes an actin-

depolymerising factor homology (ADF-H) domain and an actin binding domain. Drebrin E1 contains no inserts, E2 contains insert 1 and A contains both inserts 1 and 2. The Homer binding motif 2 is not present in chick (PQPVF at corresponding site).

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dendritic spine plasticity (Biou et al., 2008). However, in the case of both Cx43 and Ras, the precise region of Drebrin responsible for this binding is still unknown.

E1, E2 and A differ only in their short insert regions (Fig. 1), yet whether these correspond to distinct intracellular functions is unknown. One plausible scenario is that the insert fragments cause conformational changes which may expose or hide particular interaction surfaces. Another, not mutually exclusive, possibility is that key binding partners are themselves spatiotemporally regulated during development and this helps confer specificity. In fact, relatively little is known about any specific function of the embryonic isoforms. In the chick embryo, Drebrin E1 and E2 are temporally regulated with their expression corresponding to distinct phases in neuronal development. Broadly speaking, the earliest embryonic isoform, E1, is postulated to function in migration, while the E2 isoform, which replaces E1 during embryogenesis, is believed to play a role in migration as well as in the morphogenesis of axons and dendrites (Shirao and Obata 1986). Whether mammalian Drebrin E2, in the supposed absence of an E1 isoform, performs the functions of both chick E1 and E2 has yet to be determined. Throughout most of the adult rat brain, E2 is

present at consistently low levels. The exception is the rostral migratory stream which constitutes an ongoing neurogenic pathway from the subventricular zone to supply the adult olfactory bulb with new neurons. The expression of Drebrin E2 disappears immediately upon the arrival of neurons in the olfactory bulb, which further supports a role in neuronal migration and maturation (Song et al., 2008). Little is known about how the absolute and relative levels of expression of Drebrin isoforms are regulated. Within the brain, the Drebrin gene has been identified as a target of NXF (Ooe et al., 2004), a member of the basic helix-loop-helix PAS-domain (bHLH-PAS) family of transcription factors; there are several NXF binding elements within the Drebrin promoter. The spatiotemporal expression pattern of NXF mRNA overlaps with that of Drebrin A but not the embryonic isoforms. Sim2, another bHLH-PAS transcription factor has been proposed as a repressor of Drebrin expression and may compete for the same DNA binding sites as NXF (Ooe et al., 2004). The homeodomain transcription factor Pax3 can also significantly inhibit Drebrin expression (Mayanil et al., 2001). In contrast, nothing is known about how the expression of distinct embryonic Drebrin isoforms is activated nor how their alternative splicing is regulated. A possible clue may come from kidney podocyte maturation: Drebrin E2 is expressed by murine podocytes during glomerular development but is turned off in adult cells, however, Drebrin E2 has been found in cultured adult murine podocytes (Peitsch et al., 2003). Comparison of transcription factor repertoires between these instances may identify those responsible for the activation or repression of Drebrin expression.

Drebrin in synaptic function and correlated neurological diseases

Drebrin A is present in the mature neuron at excitatory synapses (Aoki et al., 2005) and its expression correlates temporally with the onset of maturation of the neuronal network, notably the terminal differentiation of synaptic connections between axons and dendrites. The proportion of dendritic spines immunoreactive for Drebrin A in the adult rat cerebral cortex then remains fairly constant, at about 75% (Aoki et al., 2005). Suppression of Drebrin A expression reduces spine density and results in the formation of thin immature spines (Takahashi et al., 2005). The shorter adult isoform, s-Drebrin A, could regulate the functions of its longer counterpart by competing for actin-binding sites (Jin et al., 2002). These findings indicate that the Drebrin-actin complex plays a pivotal role in the regulation of spine morphology and that Drebrin A has a critical role in synaptic plasticity and cognition. Drebrin forms part of a large postsynaptic protein complex which incorporates proteins related to the cytoskeleton and to synaptic transmission, including NMDA receptor subunits, CaM kinase II (CaMKII), cofilin, PSD-95, and actin (Takahashi et al., 2003; Sekino et al., 2005; Kojima

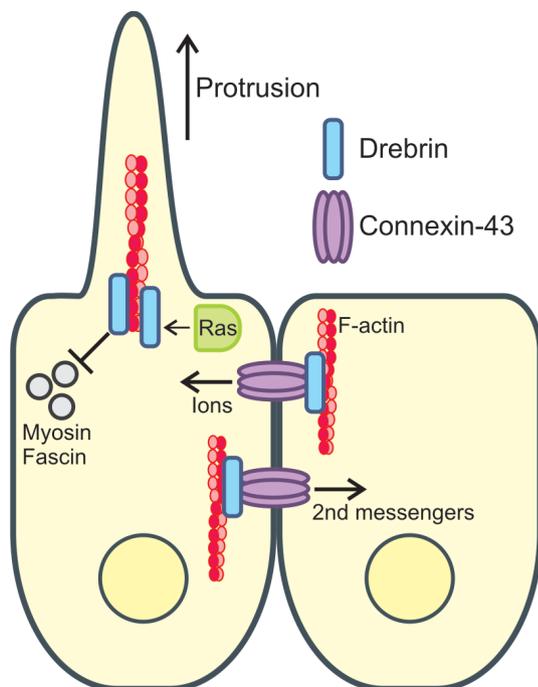


Fig. 2. Dynamics of Drebrin in motile cells. Drebrin, myosin IIB, fascin, tropomyosin and α -actinin compete for binding to F-actin in filopodia. Drebrin can inhibit the bundling activity of fascin and tropomyosin and the crosslinking activity of α -actinin; this may stabilise filopodia and help direct cell migration in response to environmental signals. The interaction with Connexin-43 can mediate cell-cell communication by the exchange of ions and second messengers, which in turn may also promote cell migration.

and Shirao 2007). The interactions between Drebrin and the constituent proteins of this complex are manifold and imply the existence of interdependent regulatory loops. Enrichment of Drebrin within spines is required to induce the accumulation of the glutamate receptor scaffolding protein, PSD-95, at postsynaptic sites *in vitro* (Takahashi et al., 2003), in turn, activation of AMPA receptors promotes Drebrin clustering (Takahashi et al., 2009). Conversely, activation of NMDA receptors induces the translocation of Drebrin from dendritic spines to dendritic shafts whilst *in vivo* blockade of NMDA receptors increases the proportion of Drebrin in dendritic spines (Sekino et al., 2005; Fujisawa et al., 2006). However, it is unclear how this relates to data showing that down-regulation of Drebrin A suppresses synaptic targeting of NMDA receptors (Takahashi et al., 2005). Nevertheless, these data support the existence of distinct feedback loops between Drebrin and different types of glutamate receptor to provide tight regulation of dendritic spine morphology and plasticity (Fig. 3). Knockdown of Drebrin A in mice produces cognitive deficits including impaired pre-pulse inhibition, increased locomotor activity, anxiety-like behaviour and

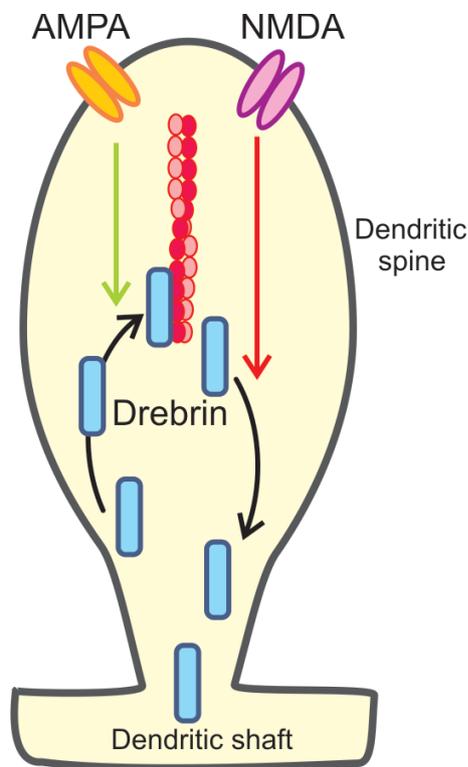


Fig. 3. Putative pathway linking Drebrin to glutamate receptors on dendritic spines. Activation of NMDA receptors induces the translocation of Drebrin from dendritic spines to dendritic shafts whilst blockade of NMDA receptors increases the proportion of Drebrin in dendritic spines. In contrast, activation of AMPA receptors promotes Drebrin recruitment and clustering.

increased sensitivity to psychostimulants, thereby drawing parallels with schizophrenia (Kobayashi et al., 2004). Furthermore, the antidepressant fluoxetine can up-regulate levels of Drebrin A mRNA in the hippocampus of chronically stressed rats (Yang et al., 2003). There is thus strong evidence that Drebrin is intimately involved in synaptic remodelling in a range of neurological functions.

Alzheimer's disease and Down Syndrome

An association of reduced levels of Drebrin with Alzheimer's disease (AD) was first reported in 1996 (Harigaya et al., 1996) and has since been confirmed by other groups using both immunohistochemistry and immunoblotting (Hatanpaa et al., 1999; Shim and Lubec 2002; Calon et al., 2004). Drebrin protein levels were found to be decreased by 70-95% in the hippocampus and frontal and temporal cortex of AD patients compared to controls. In the temporal cortex of AD patients, PSD-95 was found to be decreased by 50%; this may be secondary to Drebrin loss because antisense suppression of Drebrin expression prevents PSD-95 clustering at synapses (Takahashi et al., 2003). Consistent with these results, a 60% reduction in Drebrin is observed in the cerebral cortex of Tg2576 mice, an AD animal model. The loss is exacerbated to 90% after deprivation of docosahexaenoic acid (DHA), an essential omega-3 polyunsaturated fatty acid (Calon et al., 2004). DHA depletion also decreases NMDA receptor density in the brains of Tg2576 mice (Calon et al., 2005). Given that NMDA receptors and Drebrin generally appear to have an antagonistic relationship, their concomitant loss suggests that DHA acts upon a core synaptic component required by both NMDA receptors and Drebrin. Genetically induced reduction of PSD-95 causes cognitive deficits (Migaud et al., 1998), as does direct inactivation of Drebrin using intracerebral administration of antisense oligonucleotides, further demonstrating the critical role of Drebrin in synapse function and cognition (Kobayashi et al., 2004). The production and accumulation of β -amyloid ($A\beta$) peptide is closely linked to the pathogenesis of Alzheimer's Disease. One result of $A\beta$ oligomer aggregation is a decrease in p21-activated kinase (PAK) activity which is also observed in the temporal cortex of AD patients. PAK phosphorylates cofilin to inhibit its binding to actin where it competes with Drebrin. Reduced PAK activity in AD patients and thus less phosphorylation of cofilin could limit Drebrin binding to actin resulting in the translocation of Drebrin to the cytosol. Critically, treatment of transgenic mice with an anti- $A\beta$ antibody restored levels of PAK activity and Drebrin (Zhao et al., 2006).

A decrease in Drebrin level is also observed in Down Syndrome (DS) both from adult and foetal brain samples (Weitzdoerfer et al., 2001; Shim and Lubec 2002). DS is the most common chromosomal disorder,

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occurring in approximately 1 in 700 live births, and is caused by trisomy of chromosome 21. The phenotypic and molecular analysis of rare patients with partial trisomy 21 has led to the definition of a region of chromosome 21, the Down Syndrome critical region (DSCR), which is critical for the pathogenesis of DS. The duplication of this region is associated with many features of the disease: short stature, joint hyperlaxity, hypotonia; morphological anomalies of the face, hand, and foot; and particularly significantly in terms of Drebrin, mental retardation (Rahmani et al., 1989).

Individuals with DS frequently develop characteristics of AD, including neuritic plaques and neurofibrillary tangles. A β , the major component of neuritic plaques, is derived from β -amyloid precursor protein (APP) by the sequential cleavages of β - and γ -secretase at the β - and γ -site, respectively. The genes of both APP and BACE2 (β -site APP cleaving enzyme 2) are located in the DSCR. As described above, the production of A β oligomers could explain the loss of Drebrin in DS patients who develop AD. However, Drebrin loss is actually observed long before, in the foetal brain of DS patients (Weitzdoerfer et al., 2001). The Sim2 gene sits within the DSCR and has been proposed as a causal factor in the pathogenesis of DS (Dahmane et al., 1995; Fan et al., 1996; Yamaki et al., 1996). Excessive inhibition of Drebrin transcription by the extra copies of Sim2 might be a key component of DS (Ooe et al., 2004). Further investigation of the reasons for Drebrin loss in both AD and DS patients, at the transcriptional and post-transcriptional levels, will deepen understanding of the pathogenesis of the respective disorders.

Drebrin in non-neuronal cells

Since its initial isolation in neuronal cells, Drebrin has been identified in diverse non-neuronal cells, predominantly in association with cell processes and intercellular junctions, where it localizes in, or near, actin-rich lamellipodia and filopodia (Keon et al., 2000; Peitsch et al., 2001, 2005, 2006). This has opened new avenues of research into the roles of Drebrin in normal function and disease of the kidney, stomach, lung and skin.

In 2000, Keon et al. reported the presence of Drebrin E2 in non-neuronal adult organs (Keon et al., 2000). The highest amounts were found in the stomach and kidney, lesser amounts of protein were detected in the colon and urinary bladder, whilst trace amounts could be detected in heart, lung, liver, and epididymis. They cloned Drebrin cDNA from murine stomach tissue and verified the cell-type specific expression and sub-cellular localisation of Drebrin in stomach and kidney epithelia. In the stomach, Drebrin accumulates at the extended apical membrane of the acid-secreting parietal cells of the fundic glands. Overexpression of the Drebrin E2 isoform in cultured epithelial cells resulted in a similar

phenotype to that produced in neurons and fibroblasts (Keon et al., 2000). Thus it seems that E2 plays a similar role in actin plasticity in non-neuronal cells to that which it plays in neurons. Further characterisation in renal glomeruli indicated that Drebrin is expressed in mesangial cells and enriched in the foot processes of human and bovine (although not murine) podocytes (Peitsch et al., 2003). However, Drebrin can be detected in the primordial podocytes of early and intermediate stages of glomerular development in murine embryos as well as cultured murine podocytes *in vitro*. There are striking similarities between the highly-specialised elongations, enriched with Drebrin, characteristic of both neurons and podocytes. It is tempting to speculate that at a fundamental, cellular level, Drebrin has a generic role to produce and maintain these morphological features (Fig. 2). As cytoskeletal changes in mesangial cells and podocytes, especially the disassembly of actin microfilaments, have been reported as characteristic of the pathogenesis of a number of glomerular diseases, including diabetic glomerulopathy (Kikkawa et al., 1986; Cortes et al., 2000), it is important to investigate the potential involvement of Drebrin. If indeed there are common molecular mechanisms at play then information gained in the study of Drebrin during renal dysfunction could produce novel insights into neurological disorders and vice versa. Drebrin is found in the cell processes of septal myofibroblast-like cells during alveolar maturation in rat lung. The transient expression of Drebrin in the myofibroblasts suggests that the formation of cellular projections is related to the elongation of the secondary septum (Yamada et al., 2005). Thus, Drebrin appears to have key roles in the maturation of multiple organs during development and subsequently in the maintenance of their normal adult function by mediating specific requirements for actin plasticity.

Drebrin is enriched in the ducts of eccrine sweat glands, especially along their cell-cell boundaries and at the intercellular junctions of hair follicles, both in the bulbs and the outer root sheath (Peitsch et al., 2005). This is particularly interesting given the association of Drebrin with Connexin-43 and it seems, therefore, that Drebrin has an important role to play at the interface between cells throughout the body. Given the ability of Drebrin to form long, actin-rich protrusions, promote cell migration and cell-cell contact, it is not surprising that Drebrin is upregulated in a range of carcinomas and other tumours (Peitsch et al., 2005). Moreover, the carboxy terminal of Drebrin is thought to interact with the Ras oncogene product (Biou et al., 2008). What is unclear in terms of tumorigenesis is whether the dysregulation of Drebrin is a primary cause or secondary to other genetic or environmental insults. This parallels our lack of understanding of how Drebrin is involved in neurological disease as well: are abnormal levels of Drebrin the underlying cause or a pathological marker of other changes.

Conclusion

The spatiotemporal expression pattern of distinct Drebrin isoforms in diverse organs and its ability to regulate actin plasticity, direct cell migration and promote cell-cell contact indicate the biological significance of Drebrin from development through to the maintenance of normal, adult physiological function. Drebrin has a striking effect on cell shape and plasticity; it is perhaps not surprising that most data pertaining to its role have been obtained from cells with elaborate morphologies. In many cells, form and function are interdependent and Drebrin appears to play a fundamental role in fostering this link across a variety of tissues. Current data strongly implicate Drebrin in a number of developmental processes in which the shape of cells and their interaction with neighbouring cells is vitally important and in a range of diseases in which this balance is lost. To date, there are no published reports of Drebrin knock-out mice; the use of conditional transgenics may help elucidate its role in distinct organs and disease pathologies. Further studies are required to understand how Drebrin itself is regulated at the transcriptional and translational levels, in order for it to exert its effect in a controlled and appropriate manner.

References

- Aoki C., Sekino Y., Hanamura K., Fujisawa S., Mahadomrongkul V., Ren Y. and Shirao T. (2005). Drebrin A is a postsynaptic protein that localizes in vivo to the submembranous surface of dendritic sites forming excitatory synapses. *J. Comp. Neurol.* 483, 383-402.
- Biou V., Brinkhaus H., Malenka R.C. and Matus A. (2008). Interactions between drebrin and Ras regulate dendritic spine plasticity. *Eur. J. Neurosci.* 27, 2847-2859.
- Butkevich E., Hulsmann S., Wenzel D., Shirao T., Duden R. and Majoul I. (2004). Drebrin is a novel connexin-43 binding partner that links gap junctions to the submembrane cytoskeleton. *Curr. Biol.* 14, 650-658.
- Calon F., Lim G.P., Morihara T., Yang F., Ubada O., Salem N. Jr, Frautschy S.A. and Cole G.M. (2005). Dietary n-3 polyunsaturated fatty acid depletion activates caspases and decreases NMDA receptors in the brain of a transgenic mouse model of Alzheimer's disease. *Eur. J. Neurosci.* 22, 617-626.
- Calon F., Lim G.P., Yang F., Morihara T., Teter B., Ubada O., Rostaing P., Triller A., Salem N. Jr, Ashe K.H., Frautschy S.A. and Cole G.M. (2004). Docosahexaenoic acid protects from dendritic pathology in an Alzheimer's disease mouse model. *Neuron* 43, 633-645.
- Cheng X.T., Hayashi K. and Shirao T. (2000). Non-muscle myosin IIB-like immunoreactivity is present at the drebrin-binding cytoskeleton in neurons. *Neurosci. Res.* 36, 167-173.
- Chew C.S., Okamoto C.T., Chen X. and Thomas R. (2005). Drebrin E2 is differentially expressed and phosphorylated in parietal cells in the gastric mucosa. *Am. J. Physiol. Gastrointest. Liver Physiol* 289, G320-G331.
- Cina C., Maass K., Theis M., Willecke K., Bechberger J.F. and Naus C.C. (2009). Involvement of the cytoplasmic C-terminal domain of connexin43 in neuronal migration. *J. Neurosci.* 29, 2009-2021.
- Cortes P., Mendez M., Riser B.L., Guerin C.J., Rodriguez-Barbero A., Hassett C. and Yee J. (2000). F-actin fiber distribution in glomerular cells: structural and functional implications. *Kidney Int.* 58, 2452-2461.
- Dahmane N., Charron G., Lopes C., Yaspo M.L., Maunoury C., Decorte L., Sinet P.M., Bloch B. and Delabar J.M. (1995). Down syndrome-critical region contains a gene homologous to *Drosophila sim* expressed during rat and human central nervous system development. *Proc. Natl. Acad. Sci. USA* 92, 9191-9195.
- Fagni L., Worley P.F. and Ango F. (2002). Homer as both a scaffold and transduction molecule. *Sci. STKE.* 2002, RE8.
- Fan C.M., Kuwana E., Bulfone A., Fletcher C.F., Copeland N.G., Jenkins N.A., Crews S., Martinez S., Puellas L., Rubenstein J.L. and Tessier-Lavigne M. (1996). Expression patterns of two murine homologs of *Drosophila single-minded* suggest possible roles in embryonic patterning and in the pathogenesis of Down syndrome. *Mol. Cell Neurosci.* 7, 1-16.
- Friedl P. and Gilmour D. (2009). Collective cell migration in morphogenesis, regeneration and cancer. *Nat. Rev. Mol. Cell Biol.* 10, 445-457.
- Fujisawa S., Shirao T. and Aoki C. (2006). In vivo, competitive blockade of n-methyl-d-aspartate receptors induces rapid changes in filamentous actin and drebrin a distributions within dendritic spines of adult rat cortex. *Neuroscience* 140, 1177-1187.
- Geraldo S., Khazada U.K., Parsons M., Chilton J.K. and Gordon-Weeks P.R. (2008). Targeting of the F-actin-binding protein drebrin by the microtubule plus-tip protein EB3 is required for neuritogenesis. *Nat. Cell Biol.* 10, 1181-1189.
- Harigaya Y., Shoji M., Shirao T. and Hirai S. (1996). Disappearance of actin-binding protein, drebrin, from hippocampal synapses in Alzheimer's disease. *J. Neurosci. Res.* 43, 87-92.
- Hatanpaa K., Isaacs K.R., Shirao T., Brady D.R. and Rapoport S.I. (1999). Loss of proteins regulating synaptic plasticity in normal aging of the human brain and in Alzheimer disease. *J. Neuropathol. Exp. Neurol.* 58, 637-643.
- Hayashi K., Ishikawa R., Kawai-Hirai R., Takagi T., Taketomi A. and Shirao T. (1999). Domain analysis of the actin-binding and actin-remodeling activities of drebrin. *Exp. Cell Res.* 253, 673-680.
- Ishikawa R., Hayashi K., Shirao T., Xue Y., Takagi T., Sasaki Y. and Kohama K. (1994). Drebrin, a development-associated brain protein from rat embryo, causes the dissociation of tropomyosin from actin filaments. *J. Biol. Chem.* 269, 29928-29933.
- Jin M., Tanaka S., Sekino Y., Ren Y., Yamazaki H., Kawai-Hirai R., Kojima N. and Shirao T. (2002). A novel, brain-specific mouse drebrin: cDNA cloning, chromosomal mapping, genomic structure, expression, and functional characterization. *Genomics* 79, 686-692.
- Keon B.H., Jedrzejewski P.T., Paul D.L. and Goodenough D.A. (2000). Isoform specific expression of the neuronal F-actin binding protein, drebrin, in specialized cells of stomach and kidney epithelia. *J. Cell Sci.* 113 Pt 2, 325-336.
- Kikkawa R., Kitamura E., Fujiwara Y., Arimura T., Haneda M. and Shigeta Y. (1986). Impaired contractile responsiveness of diabetic glomeruli to angiotensin II: a possible indication of mesangial dysfunction in diabetes mellitus. *Biochem. Biophys. Res. Commun.* 136, 1185-1190.
- Kobayashi R., Sekino Y., Shirao T., Tanaka S., Ogura T., Inada K. and Saji M. (2004). Antisense knockdown of drebrin A, a dendritic spine protein, causes stronger preference, impaired pre-pulse inhibition,

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- and an increased sensitivity to psychostimulant. *Neurosci. Res.* 49, 205-217.
- Kojima N. and Shirao T. (2007). Synaptic dysfunction and disruption of postsynaptic drebrin-actin complex: a study of neurological disorders accompanied by cognitive deficits. *Neurosci. Res.* 58, 1-5.
- Kojima N., Kato Y., Shirao T. and Obata K. (1988). Nucleotide sequences of two embryonic drebrins, developmentally regulated brain proteins, and developmental change in their mRNAs. *Brain Res.* 464, 207-215.
- Kojima N., Shirao T. and Obata K. (1993). Molecular cloning of a developmentally regulated brain protein, chicken drebrin A and its expression by alternative splicing of the *drebrin* gene. *Brain Res. Mol. Brain Res.* 19, 101-114.
- Lecuit T. and Lenne P.F. (2007). Cell surface mechanics and the control of cell shape, tissue patterns and morphogenesis. *Nat. Rev. Mol. Cell Biol.* 8, 633-644.
- Mammoto A., Sasaki T., Asakura T., Hotta I., Imamura H., Takahashi K., Matsuura Y., Shirao T. and Takai Y. (1998). Interactions of drebrin and gephyrin with profilin. *Biochem. Biophys. Res. Commun.* 243, 86-89.
- Mayanil C.S., George D., Freilich L., Miljan E.J., Mania-Farnell B., McLone D.G. and Bremer E.G. (2001). Microarray analysis detects novel Pax3 downstream target genes. *J. Biol. Chem.* 276, 49299-49309.
- Migaud M., Charlesworth P., Dempster M., Webster L.C., Watabe A.M., Makhinson M., He Y., Ramsay M.F., Morris R.G., Morrison J.H., O'Dell T.J. and Grant S.G. (1998). Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. *Nature* 396, 433-439.
- Mizui T., Takahashi H., Sekino Y. and Shirao T. (2005). Overexpression of drebrin A in immature neurons induces the accumulation of F-actin and PSD-95 into dendritic filopodia, and the formation of large abnormal protrusions. *Mol. Cell Neurosci.* 30, 149-157.
- Ooe N., Saito K., Mikami N., Nakatuka I. and Kaneko H. (2004). Identification of a novel basic helix-loop-helix-PAS factor, NXF, reveals a Sim2 competitive, positive regulatory role in dendritic-cytoskeleton modulator drebrin gene expression. *Mol. Cell Biol.* 24, 608-616.
- Peitsch W.K., Bulkescher J., Spring H., Hofmann I., Goerdt S. and Franke W.W. (2006). Dynamics of the actin-binding protein drebrin in motile cells and definition of a juxtannuclear drebrin-enriched zone. *Exp. Cell Res.* 312, 2605-2618.
- Peitsch W.K., Hofmann I., Bulkescher J., Hergt M., Spring H., Bleyl U., Goerdt S. and Franke W.W. (2005). Drebrin, an actin-binding, cell-type characteristic protein: induction and localization in epithelial skin tumors and cultured keratinocytes. *J. Invest Dermatol.* 125, 761-774.
- Peitsch W.K., Hofmann I., Endlich N., Pratzel S., Kuhn C., Spring H., Grone H.J., Kriz W. and Franke W.W. (2003). Cell biological and biochemical characterization of drebrin complexes in mesangial cells and podocytes of renal glomeruli. *J. Am. Soc. Nephrol.* 14, 1452-1463.
- Peitsch W.K., Hofmann I., Pratzel S., Grund C., Kuhn C., Moll I., Langbein L. and Franke W.W. (2001). Drebrin particles: components in the ensemble of proteins regulating actin dynamics of lamellipodia and filopodia. *Eur. J. Cell Biol.* 80, 567-579.
- Rahmani Z., Blouin J.L., Creau-Goldberg N., Watkins P.C., Mattei J.F., Poissonnier M., Prieur M., Chettouh Z., Nicole A., Aurias A., Sinet P. and Delabar J. (1989). Critical role of the D21S55 region on chromosome 21 in the pathogenesis of Down syndrome. *Proc. Natl. Acad. Sci. USA* 86, 5958-5962.
- Sasaki Y., Hayashi K., Shirao T., Ishikawa R. and Kohama K. (1996). Inhibition by drebrin of the actin-bundling activity of brain fascin, a protein localized in filopodia of growth cones. *J. Neurochem.* 66, 980-988.
- Sekino Y., Tanaka S., Hanamura K., Yamazaki H., Sasagawa Y., Xue Y., Hayashi K. and Shirao T. (2005). Activation of N-methyl-D-aspartate receptor induces a shift of drebrin distribution: Disappearance from dendritic spines and appearance in dendritic shafts. *Mol. Cell Neurosci.* 31, 493-504.
- Shim K.S. and Lubec G. (2002). Drebrin, a dendritic spine protein, is manifold decreased in brains of patients with Alzheimer's disease and Down syndrome. *Neurosci. Lett.* 324, 209-212.
- Shiraishi-Yamaguchi Y., Sato Y., Sakai R., Mizutani A., Knopfel T., Mori N., Mikoshiba K. and Furuichi T. (2009). Interaction of Cupidin/Homer2 with two actin cytoskeletal regulators, Cdc42 small GTPase and Drebrin, in dendritic spines. *BMC. Neurosci.* 10, 25.
- Shirao T., Hayashi K., Ishikawa R., Isa K., Asada H., Ikeda K. and Uyemura K. (1994). Formation of thick, curving bundles of actin by drebrin A expressed in fibroblasts. *Exp. Cell Res.* 215, 145-153.
- Shirao T. and Obata K. (1986). Immunohistochemical homology of 3 developmentally regulated brain proteins and their developmental change in neuronal distribution. *Brain Res.* 394, 233-244.
- Shirao T., Kojima N., Kato Y. and Obata K. (1988). Molecular cloning of a cDNA for the developmentally regulated brain protein, drebrin. *Brain Res.* 464, 71-74.
- Song M., Kojima N., Hanamura K., Sekino Y., Inoue H.K., Mikuni M. and Shirao T. (2008). Expression of drebrin E in migrating neuroblasts in adult rat brain: coincidence between drebrin E disappearance from cell body and cessation of migration. *Neuroscience* 152, 670-682.
- Takahashi H., Sekino Y., Tanaka S., Mizui T., Kishi S. and Shirao T. (2003). Drebrin-dependent actin clustering in dendritic filopodia governs synaptic targeting of postsynaptic density-95 and dendritic spine morphogenesis. *J. Neurosci.* 23, 6586-6595.
- Takahashi H., Mizui T. and Shirao T. (2005). Down-regulation of drebrin A expression suppresses synaptic targeting of NMDA receptors in developing hippocampal neurones. *J. Neurochem.* 97, 110-115.
- Takahashi H., Yamazaki H., Hanamura K., Sekino Y. and Shirao T. (2009). Activity of the AMPA receptor regulates drebrin stabilization in dendritic spine morphogenesis. *J. Cell Sci.* 122, 1211-1219.
- Toda M., Shirao T., Minoshima S., Shimizu N., Toya S. and Uyemura K. (1993). Molecular cloning of cDNA encoding human drebrin E and chromosomal mapping of its gene. *Biochem. Biophys. Res. Commun.* 196, 468-472.
- Toda M., Shirao T. and Uyemura K. (1999). Suppression of an actin-binding protein, drebrin, by antisense transfection attenuates neurite outgrowth in neuroblastoma B104 cells. *Brain Res. Dev. Brain Res.* 114, 193-200.
- Weitzdoerfer R., Dierssen M., Fountoulakis M. and Lubec G. (2001). Fetal life in Down syndrome starts with normal neuronal density but impaired dendritic spines and synaptosomal structure. *J. Neural Transm. Suppl.* 59-70.
- Yamada M., Kurihara H., Kinoshita K. and Sakai T. (2005). Temporal expression of alpha-smooth muscle actin and drebrin in septal interstitial cells during alveolar maturation. *J. Histochem. Cytochem.* 53, 735-744.

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- Yamaki A., Noda S., Kudoh J., Shindoh N., Maeda H., Minoshima S., Kawasaki K., Shimizu Y. and Shimizu N. (1996). The mammalian single-minded (SIM) gene: mouse cDNA structure and diencephalic expression indicate a candidate gene for Down syndrome. *Genomics* 35, 136-143.
- Yang H.J., Li Y.F., Zhang H.T., Zhang F.Q., Zhao N., Gong Z.H. and Luo Z.P. (2003). Up-regulation of microtubule-associated protein 4 and drebrin A mRNA expression by antidepressants in rat hippocampus following chronic stress. *Neurosci. Lett.* 351, 206-208.
- Zhao L., Ma Q.L., Calon F., Harris-White M.E., Yang F., Lim G.P., Morihara T., Ubeda O.J., Ambegaokar S., Hansen J.E., Weisbart R.H., Teter B., Frautschy S.A. and Cole G.M. (2006). Role of p21-activated kinase pathway defects in the cognitive deficits of Alzheimer disease. *Nat. Neurosci.* 9, 234-242.

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