CELL SIGNALLING

New tricks for DIX

The 85-residue DIX protein domain is rather mysterious. Although it's present in a family of eukaryotic signalling proteins, including dishevelled (Dvl), its structure and function have remained unclear. In *Nature*, however, Michael Overduin and colleagues now reveal that DIX has a role in segregating Dvl into two different intracellular pools, and that, by doing so, it controls a crucial divergence point in the Wnt signalling pathway.

Dvl signals downstream of Wnt receptors in the Wnt signalling pathway. Membrane-targeted Dvl stabilizes β-catenin and thus Wnt signalling, which promotes cell proliferation and embryonic axis formation.

In Chinese hamster ovary (CHO) cells, Overduin and co-workers saw that endogenous dishevelled-2 (Dvl2) exists in two pools — one associated with actin stress fibres and another associated with cytoplasmic vesicles. To investigate whether the DIX domain is involved in Dvl targeting, the authors overexpressed the Dvl2 DIX domain and full-length Dvl2 in CHO cells, and, in both cases, they found that Dvl2 co-localized with actin and cytoplasmic vesicles. However, when they overexpressed a Dvl2 construct that lacked the DIX domain (Dvl2ΔDIX), they found that it remained free in the cytosol.

Using NMR techniques, the authors showed that the Dvl2 DIX domain has two α-helices (α1 and α2), and helical (α3) and extended structural elements, although their three-dimensional arrangement remains unknown. Furthermore, they identified an actin-binding element — YFFKSM^{60} — next to α2 of the Dvl2 DIX domain, as well as a phospholipid-interaction loop — VKEEIS^{72} — between the actin-binding element and α3. They found that mutating lysine 58 in the actin-binding motif to alanine (K58A) abolished actin binding, and, in addition, showed that mutating lysine 68 and glutamate 69 of the phospholipid-binding region to alanine (K68A/E69A) disrupted vesicle associations, without disrupting actin binding.

So, how is this relevant to Wnt signalling? The authors compared the levels of β-catenin in CHO cells that were overexpressing wild-type Dvl2, Dvl2ΔDIX and Dvl2(K68A/E69A), and found that they were significantly lower in the latter two cases. This result is consistent with the inability of Dvl2ΔDIX and Dvl2(K68A/E69A) to be targeted to membranes, and hence to stabilize β-catenin. When they compared the levels of β-catenin in CHO cells overexpressing wild-type Dvl2 and Dvl2(K58A), they found no difference, which indicates that the actin-binding property of Dvl2 is not required for Wnt signalling.

In *Xenopus*, signalling by β-catenin activates genes that are needed for dorsal axis formation. When the authors studied Dvl2(K58A)-mutant *Xenopus* axis induction, they found that the dorsal axis duplication frequency was increased, which indicates that the actin-bound pool of Dvl2 is usually sequestered from downstream Wnt/β-catenin signalling. By contrast, they showed that axis duplication was not induced in Dvl2(K68A/E69A)-mutant *Xenopus*, which indicates that vesicle targeting of Dvl2 is needed for downstream Wnt/β-catenin signalling. Overduin and colleagues have therefore shown that the DIX domain is a new signalling module that can target proteins to actin stress fibres and cytoplasmic vesicles to control Wnt signalling.

Rachel Smallridge

References and links

ORIGINAL RESEARCH PAPER Capelluto, D. G. S. *et al.* The DIX domain targets dishevelled to actin stress fibres and vesicular membranes. *Nature* **419**, 726–729 (2002)

WEB SITES

Michael Overduin's laboratory: http://www2.uchsc.edu/pharm/Faculty/overduin.asp

Encyclopedia of Life Sciences: http://www.els.net

Signal transduction pathways in development: Wnts and their receptors

CANCER

Filling the hole

A hollow glandular architecture is associated with many highly organized tissues such as the mammary gland, and apoptosis functions to create this space. Now, the authors of a recent *Cell* paper have used non-transformed MCF-10A mammary epithelial cells in a three dimensional cell-culture model — a system in which the cells can take on many of the *in vivo* features of breast epithelium — to show that apoptosis is important in maintaining luminal space and that tumour cells must suppress apoptosis to successfully invade the lumen.

On studying cell death during acinar morphogenesis, the authors observed, after 5–8 days in culture, a well polarized outer layer of cells surrounding a subset of poorly polarized cells. The cells in the interior of the structure — the presumptive luminal space — died after 6–8 days, just before the lumen appeared. A lack of survival-promoting signals from the Akt pathway was implicated in this cell death, which proceeded through a caspase-dependent mechanism.

To determine whether lumen formation can occur in the absence of apoptosis, the authors overexpressed the anti-apoptotic proteins Bcl-2 and Bcl-X_L. Although lumen formation was delayed, cells were eventually cleared — possibly by autophagy — and a luminal space formed. Conversely, and perhaps somewhat surprisingly, increased proliferation, which the authors induced by overexpressing cyclin D1 or human papilloma virus (HPV) 16 E7, did not fill the luminal space either. Large amounts of cellular debris and fragmented nuclei provided clues as to why this was — increased cell death was occurring.

So this begs the obvious question as to how certain oncogenes can cause cells to invade the luminal space, which occurs in early epithelial tumours. To address this, the authors cultured MCF-10A cells stably expressing either cyclin D1 with Bcl-X_L, or HPV 16 E7 with Bcl-2, and found that the luminal space of acini in which both the proliferative and anti-apoptotic genes were simultaneously expressed became filled.

It seems, therefore, that only a combination of increased proliferation and decreased cell death can fill the lumen; 'isolated' biological insults induced by many cancer genes have little effect on disrupting epithelial architecture. Notably, activated ErbB2, which induces both of these biological activities, can fill the luminal space in this model system; it's also overexpressed in many metastatic breast cancers.

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FURTHER READING O'Brien, L. E. *et al.* Building epithelial architecture: insights from three-dimensional culture models. *Nature Rev. Mol. Cell Biol.* **3**, 531–537 (2002) | Muthuswamy, S. K. *et al.* ErbB2, but not ErbB1, reinitiates proliferation and induces luminal repopulation in epithelial acini. *Nature Cell Biol.* **3**, 785–792 (2001)