RESEARCH HIGHLIGHTS

Phosphatidylinositol control of endocytosis

During clathrin-mediated endocytosis (CME), the phosphoinositide phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P₂) recruits proteins involved in the formation of clathrin-coated pits (CCPs). During subsequent endosomal stages, PtdIns(3)P predominates. Investigating how such phosphatidylinositol switching is achieved, Haucke and colleagues (Nature 499, 233-237; 2013) have identified a previously uncharacterized function for PtdIns(3,4)P, in CME. They showed that depleting this lipid or the class II PtdIns(3) kinase C2a (PI(3)K C2a, which phosphorylates PtdIns(4)P) from CCPs, inhibited transferrin CME and led to increased transferrin receptor surface levels. Furthermore, CCPs were longerlived, and quantitative morphometric analysis revealed that PI(3)K C2a was required for the transition from invaginated to omega-shaped CCPs during maturation, before dynaminmediated fission. The delay in CCP maturation caused by depleting PI(3)K C2 α or PI(3,4)P₂ prompted a search for PtdIns(3,4)P₂ effectors at CCPs, which revealed that the PX-BAR domain protein sorting nexin 9 (SNX9) bound preferentially to PtdIns(3,4)P, over PtdIns(4,5)P, in brain extracts, and failed to accumulate at latestage endocytic intermediates when PtdIns(3,4) P_2 or PI(3)K C2a was depleted from cells. These results have uncovered that PtdIns(3,4)P, functions in CME through the action of PI(3) K C2a and possibly in conjunction with PtdIns(4,5)P, phosphatases, adding to our understanding of how endocytosis is spatiotemporally controlled, and providing impetus to study this lipid in other aspects of physiology and disease. KL

Metalloproteinases in mammary stem cell activity

Adult mammary stem cells remodel the mammary gland in development and pregnancy. The mammary stem cell population expands in response to extracellular signals such as Wnt. Extracellular zinc-dependent endopeptidases of the matrix metalloproteinase (MMPs) family are thought to promote tumour cell invasion and metastasis. Werb and colleagues have found that MMP3 modulates Wnt signalling to control mammary stem cell function (Cell Stem Cell http://doi.org/10/nfh; 2013). The authors showed that overexpression in mammary stem cells of the full-length MMP3, or of its hemopexin domain (HPX) (which binds MMP substrates but has no catalytic activity), leads to their hyperplastic growth when transplanted in mouse mammary fat pads. They also demonstrated that the mammary stem cell population is decreased in MMP3-deficient mice, and that it loses some of its reconstitution properties in vivo following transplantation.

Using a yeast two-hybrid approach, they identified the non-canonical Wnt ligand, Wnt5b, as an MMP3-HPX interactor. The authors showed that Wnt5b activates a non-canonical signalling pathway that positively regulates the

Platelets assist in extravasation

Platelets can be activated by tumour cells, but how they contribute to metastasis has been unclear. Offermans and colleagues now show that platelet activation by tumour cells facilitates transendothelial cell migration (*Cancer Cell* **24**, 130–137; 2013).

By studying the transmigration of cancer cells through an endothelial cell layer *in vitro*, the authors found that cell lines derived from melanoma and lung carcinoma successfully migrated between the endothelial cells in the presence of either activated platelets or their supernatant. Degradation of platelet-derived nucleotides, such as ATP and ADP, inhibited transendothelial migration. These nucleotides are produced from dense granules that are released by active platelets. Blocking the release of these granules by using mice that lack the exocytosis-priming protein MUNC13-4 inhibited the transendothelial migration of the cancer cells from subcutaneous xenografts and after tail vein injection. The authors further showed that an adenosine receptor, P2Y₂, which is expressed on endothelial cells, is required for successful migration: mice lacking P2Y₂ in endothelial cells had reduced levels of pulmonary metastases compared with controls after tail vein injection of tumour cells. Platelet-derived ATP was found to cause a loss of tight interendothelial junctions, as shown by the leakage of labelled dextran across the lung endothelial cell barrier *in vivo*.

These findings raise the possibility that $P2Y_2$ and its associated signalling pathway could represent the represent therapeutic targets, which remains a topic for further study. NM transcription factor NFAT to inhibit mammary stem cell proliferation. However, as reported before of other non-canonical Wnt ligands, they also found that Wnt5b interferes with canonical Wnt– β -catenin signalling by occupying LRP5/6 co-receptors. Further analysis indicated that MMP3 binds to a Wnt5b domain overlapping the region that interacts with the co-receptors, providing insight into how MMP3 counteracts the action of Wnt5b to regulate mammary stem cell function. NLB

Controlling lifespan with NAD+

Nicotinamide adenine dinucleotide (NAD+) is a substrate for sirtuins, enzymes implicated in metabolism and lifespan control, and a donor molecule for polyADP ribose polymerases (PARPs), which have also been linked to metabolism. Auwerx and colleagues now report that NAD+ controls lifespan by activating stress responses, including FOXO signal-ling and the mitochondrial unfolded protein response (UPR^{mt}) (*Cell* **154**, 430–441; 2013).

The authors observed that ageing mice and Ceanorhabditis elegans had increased PARP activity and lower NAD+ levels. Inhibiting PARP activity or providing NAD+ precursors extended worm lifespan, which was dependent on the presence of the sir-2.1 sirtuin. Treatment with PARP inhibitors or NAD+ precursors also enhanced mitochondrial activity, as shown by increased respiration, mitochondrial abundance and gene expression of metabolic pathway components. Moreover, elevating NAD+ levels in worms induced an early-phase activation of the UPR^{mt} and a late-phase activation of the FOXO pathway, which is known to act as a defence response to increased reactive oxygen species. Further C. elegans lifespan analyses demonstrated that sir-2.1 expression is epistatically linked to the UPR^{mt}. Finally, the authors showed that increased NAD+ levels and sirtuin activity also induced UPRmt and FOXOdependent responses in mammalian cells.

Future studies will determine whether this evolutionarily conserved NAD+-sirtuin– UPR^{mt}-FOXO pathway could be manipulated for the treatment of ageing-related disorders. AIZ

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