RESEARCH HIGHLIGHTS

Autophagy proteins regulate bone resorption

Secretory lysosomes containing degradative enzymes such as cathepsin K fuse with the ruffled border of osteoclast plasma membranes to promote the digestion and resorption of bone material. Although genome-wide association studies have hinted at a link between autophagy and bone homeostasis, the molecular details have remained elusive. Teitelbaum, Virgin and colleagues now reveal that the autophagy machinery is involved in generation of the osteoclast ruffled border and bone degradation (*Dev. Cell* **21,** 966–974; 2011).

Autophagy is regulated by a cascade of events, including Atg7 (autophagy-related protein 7)-dependent conjugation of Atg5 to Atg12, and subsequent lipidation of LC3I (light chain 3 I) to form LC3II. The authors visualized LC3II at the ruffled border of wild-type mouse osteoclasts, but found that loss of *Atg5* blocked this localization. Moreover, deletion of *Atg5* or *Atg7* inhibited accumulation of cathepsin-K-containing lysosomes at the ruffled border, and impaired bone degradation *in vitro* and *in vivo*. Rab7, which is known to mediate ruffled border formation, was also mislocalized in *Atg5*-null osteoclasts.

These results suggest that Atg5 functions upstream of Rab7 to modulate ruffled border formation. Indeed, this membrane structure was absent or aberrantly formed in Atg5-deficient osteoclasts. The authors propose that autophagy proteins mediate fusion of secretory lysosomes with the plasma membrane to form the ruffled border and regulate bone resorption, revealing a previously unappreciated role for autophagy machinery in osteoclast polarized secretion. EJC

A pituitary gland in a dish

Endocrine cells of the mammalian pituitary gland release systemic hormones, such as adenocorticotropic hormone (ACTH), luteinizing hormone, follicle-stimulating hormone and prolactin, in response to signals from the adjacent hypothalamus. During development the gland emerges from the rostral head ectoderm, and its elaborate organisation depends on interactions with adjacent neural tissues. By partly reproducing these interactions *in vitro*, Sasai and colleagues obtained a functional pituitary gland from embryonic stem cells (*Nature* **480,** 57–62; 2011).

The authors determined that aggregation of many embryonic stem cells in the presence of BMP4 led to the simultaneous differentiation of an epithelium with characteristics of the rostral head ectoderm overlaying a hypothalamic neuroectodermal cell layer. Following addition of Hedgehog agonists (known to promote pituitary gland fate *in vivo*), hollow epithelial structures formed in which the cells adopted the polarity and organisation characteristic of the pituitary gland. Inhibiting Notch signalling in these cultures was then sufficient to induce the differentiation of distinct endocrine cell types. As observed *in vivo*, ACTH release from these cells was induced by the gonadotropin hormone and inhibited by glucocorticoids. Finally, following ablation of the pituitary gland in mice, the authors transplanted the aggregates into kidney capsules and observed restored blood ACTH levels, as well as the physiological activities dependent on this hormone. Whether these transplanted aggregates show the plasticity that characterises the pituitary gland, which dynamically changes its proportions of endocrine cells in response to physiology, remains to be explored. NLB

pH-dependent invadopodia control

Invadopodia are proteolytic actin-rich protrusions involved in cancer cell invasion. Condeelis and colleagues now identify a pH-dependent regulatory step in invadopodia maturation (*J. Cell Biol.* **28,** 903–920; 2011).

The binding of cortactin to cofilin is known to inhibit cofilin's actin-severing activity, and cortactin phosphorylation releases this inhibition in a cycle that is essential for invadopodia function. The authors used FRET analysis of invadopodia to confirm that cortactin phosphorylation correlates with reduced interaction between the proteins. However, phosphorylation of cortactin did not affect this interaction *in vitro;* it was instead reduced by an increase in buffer pH. The authors found the pH enhanced in mature invadopodia in direct correlation with cortactin intensity, and cells stable at high pH displayed increased amounts of free barbed actin ends at invadopodia.

In accordance with a role for pH in invadopodium formation, interaction of the sodium hydrogen exchanger NHE1 with cortactin was promoted by cortactin phosphorylation, and NHE1 depletion influenced the cortactin–cofilin interaction at invadopodia. Finally, 3D invasion assays revealed the requirement for cortactin phosphorylation, cofilin and NHE1 in the formation of long invadopodia, and for a dynamic invadopodia protrusion–retraction cycle.

Thus, the authors propose that cortactin phosphorylation recruits NHE1 to invadopodia, where a local increase in pH releases cofilin to activate actin barbed-end generation for invadopodia elongation. CKR

CRAF breaks up with MEK to regulate mitosis in cancer

RAF kinases are key components of the mitogen-activated protein kinase (MAPK) pathway, where they activate MEK to regulate cell proliferation. Mielgo *et al.* now report that the CRAF isoform promotes the activation of mitotic kinases in a MEK-independent manner to enhance proliferation and tumour growth (*Nat. Med.* **17,** 1641–1645; 2011).

The authors observed that CRAF deficiency led to mitotic arrest and reduced proliferation in mouse embryonic fibroblasts and cancer cells. Treatment of cancer cells with allosteric, but not ATP-competitive, RAF inhibitors resulted in a similar phenotype that was not rescued by ectopic MEK expression. This suggests that CRAF acts independently of its kinase activity and MEK activation in this context. The authors established the existence of such a pathway using CRAF mutants, and demonstrated that it depends on the phosphorylation of CRAF at Ser 388, a modification previously linked to cancer progression. They showed that Ser-388-phosphorylated CRAF accumulates at centrosomes and spindle poles, where it interacts with the Aurora-A and Plk1 mitotic kinases to enhance their activation for mitotic progression. Expression of a phosphomimetic Ser 338 CRAF mutant in cancer cell lines activated Plk1, but not MEK, and accelerated mitosis and tumour growth in mice. These findings have potential therapeutic implications for the targeting of RAF in cancer. AIZ

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