CELL SIGNALLING

From Wingless to boneless

Human bone mass is the result of a fine balancing act between bone deposition — carried out by bone-forming cells called osteoblasts — and degradation by the osteoclasts. In later life, as bone catabolism supersedes anabolism, the result is often a loss of bone mass (osteoporosis). To prevent this condition, we need to know more about the processes that underlie bone formation, and a report in *Cell* by Matthew Warman and co-workers now takes us a step closer, revealing an intriguing link to the Wingless (Wnt) signalling pathway.

Warman, along with Yaoqin Gong and an international group of clinicians and scientists, started by searching for the gene behind osteoporosis-pseudoglioma syndrome (OPPG) — an autosomal-recessive disorder in which patients have very low bone mass. They studied 28 affected families, and identified six different homozygous frameshift or nonsense mutations in the low-density lipoprotein receptor-related protein-5 (*LRP5*) gene.

Next the authors looked at expression of mouse *Lrp5* messenger RNA during embryogenesis by *in situ* hybridization on developing skeletal elements. They detected expression in osteoblasts; interestingly, in pluripotent cell lines induced into the osteoblastic lineage by exogenous growth factors, the authors also found increased expression of LRP5. And this could, they speculate, indicate a role for LRP5 in terminal osteoblastic differentiation.

To delve deeper into the function of LRP5, Warman and co-workers used what is already known about its relatives. The mouse, *Xenopus laevis* and *Drosophila melanogaster* paralogues of LRP5 are involved in the Wnt signalling cascade, so the authors examined the effects of expressing various Wnt proteins *in vitro*.

To do this they used alkaline phosphatase (ALP) as a marker of osteoblast differentiation in two pluripotent mesenchymal cell lines. Whereas WNT3a — which participates in the canonical Wnt signalling pathway — could induce ALP activity in both lines, WNT5a and WNT4 (which use other signalling pathways) could not. WNT3a-induced ALP activity was also reduced when dominant-negative forms of LRP5 that lack the cytoplasmic tail were expressed in these cell lines. Moreover, induction of ALP by WNT3a could be blocked by coexpression with a dominant-negative form of Dishevelled, which acts downstream of WNT3a in the canonical pathway.

Finally, Warman and colleagues studied the effect of culturing bone explants in conditioned media from cells expressing the secreted form of

CELL SIGNALLING

Closing the gap

Cell signalling and membrane transport have traditionally been separate fields of cell biology. But they seem to converge more and more, as illustrated by a report in *Science* that describes a new GTPase-activating protein (GAP) for $G\alpha_s$ subunits of heterotrimeric G proteins that seems to also function as a sorting nexin in controlling the lysosomal degradation of epidermal growth factor receptors (EGFRs).

Gα subunits come in many flavours, and each of them seems to be regulated by a specific GAP of the regulator of G-protein signalling (RGS) family. Farquhar and colleagues set out to find the RGS protein that regulates $G\alpha_s$ — a polyvalent Gα subunit that controls various cellular responses, including cell growth, hormone secretion, and learning and memory. They searched databases for proteins with an RGS domain, and found a sequence that contained such a domain as well as a Phox domain, so they called the protein RGS-PX1.

They showed that RGS-PX1 interacts specifically and directly with $G\alpha_s$, and that it acts as a GAP for $G\alpha_s$ *in vitro*. They also confirmed that the RGS domain of RGS-PX1 attenuates $G\alpha_s$ signalling in HEK293 cells and neonatal rat cardiac membranes.

So, the RGS domain of RGS-PX1 seemed fully functional. What about the Phox domain? Farquhar and colleagues wondered whether RGS-PX1 could function as a sorting nexin — a family of proteins characterized by a Phox domain and involved in membrane transport. They overexpressed RGS-PX1 in HEK293 cells and studied its effect on EGFR sorting. Whereas in control cells, ligand-bound EGFR was rapidly sorted to lysosomes and degraded, in RGS-PX1-expressing cells, its degradation was markedly delayed, which resulted in sustained activation of downstream targets of EGFR.

Farquhar and colleagues also determined that, like other Phox domains, the PX1 domain could bind to specific phosphoinositides, including phosphatidylinositol 3-phosphate, which is abundant in endosomes. So they tested the localization of RGS-PX1 and found that it is indeed associated with early endosomes, as it colocalized with the early endosomal marker EEA1 in COS-7 cells (see picture).

So, RGS-PX1 is both a GAP for $G\alpha_s$ and a sorting nexin involved in sorting of EGFR. This new link between membrane transport and cell signalling might help us to understand how membrane transport compartmentalizes signal transduction and, conversely, how signal transduction regulates membrane transport.

Raluca Gagescu

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LRP5, which could act as a decoy receptor. In three independent experiments they showed that these explants had lower bone mass than did explants cultured in media from cells expressing the wild-type, non-secreted form of LRP5. The authors additionally found that carriers of OPPG mutations also have reduced bone mass compared with non-carrier controls, suggesting that the activity of LRP5 is dosage sensitive.

These impressive results not only implicate LRP5 in the acquisition of bone mass, but they provide a clue as to how it does this. Given that LRP5 is expressed in several different tissues, one surprise is that the phenotypic effects of the mutation seem to appear only in the skeleton and the eye. This could mean that the functions of LRP5 are redundant or that it binds to other ligands too — questions that will need to be answered if pharmacological modulation of LRP5 is to be used in the fight against osteoporosis.

Alison Mitchell

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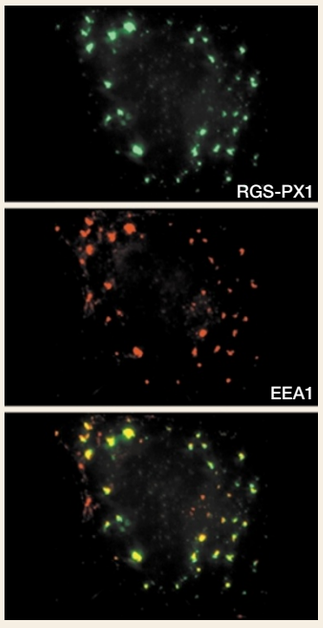
Warman laboratory: http://genetics.gene.cwru.edu/bone/



SIGNAL TRANSDUCTION

Chop and change

CD44 is an important cell-surface adhesion molecule. It is expressed in most human cell types and has been implicated in many physiological and pathological processes, such as cell migration and the regulation of tumour cell growth and metastasis. To meditate these processes, CD44 must be able to transduce numerous intracellular signals but how this occurs has remained unclear. Now, in *The Journal of Cell Biology*, Okamoto and co-workers report the identification of a novel CD44 signalling pathway.

It was previously shown that the extracellular ectodomain of CD44 can be proteolytically cleaved by membrane-associated metalloproteinases (MMPs) to produce soluble CD44 and membrane-bound CD44 ectodomain cleavage products. As this cleavage regulates the cell-migration function of CD44, Okamoto and colleagues investigated how proteolysis could affect other CD44 functions.

By inducing calcium influx or by using 12-*O*-tetradecanoylphorbol-13-acetate (TPA) treatment, the authors induced the activation of MMPs in human glioma cells. Using immunoblot analysis, they observed the expected CD44 ectodomain cleavage products, but, at a later timepoint, they also observed a smaller fragment of CD44, which corresponded to the intracellular domain (ICD). They showed that, after MMPs act to generate ectodomain cleavage products, further cleavage by intracellular proteases produces CD44ICD.

So what does CD44ICD do? In transiently transfected cells, Okamoto and co-workers showed that tagged CD44ICD is localized to the nucleus. This nuclear localization was also demonstrated for endogenous CD44ICD. Using a luciferase reporter, the authors showed that CD44ICD can enhance transcription that is mediated through the TPA-responsive element (TRE), and that CD44ICD translocation to the nucleus is essential for this enhancement. MMP inhibitors block CD44-dependent transcription enhancement and the enhancement is not observed when CD44 is mutated to remove the intracellular proteolytic cleavage site. The authors therefore concluded that sequential proteolytic cleavage of CD44 and release of CD44ICD is essential for CD44-dependent transcription enhancement.

Using GAL4 transactivation assays, the authors showed that CD44ICD alone is unlikely to act as a transcription factor. So, they tested the hypothesis that it affects other transcription factors — c-Fos and c-Jun — or transcriptional coactivators — CREB-binding protein (CBP) and p300 — involved in TRE-mediated transcription. CD44ICD did not affect GAL4–c-Fos- or GAL4–c-Jun-induced transcription from a GAL4-dependent promoter, but it did enhance transcription by GAL4–CBP and GAL4–p300. Whether the CD44ICD transactivation mediated through CBP/p300 occurs by a direct or indirect interaction remains to be determined.

To identify the endogenous gene targets of CD44ICD, the authors compared HeLa cells transfected with either a control plasmid or one encoding CD44ICD. The *CD44* gene contains TRE sequences in its promoter region, and Okamoto and co-workers found that CD44ICD induces *CD44* expression. They propose that this CD44ICD-induced *CD44* transcription promotes the rapid turnover of CD44 that is required for cell migration.

Signalling pathways are usually thought to involve interactions between cell-surface proteins and cytoplasmic proteins, which, in turn, regulate gene transcription. Here, however, Okamoto and colleagues have shown that CD44 bypasses a step in this pathway, as CD44ICD itself can activate gene transcription. In addition to identifying a novel CD44 signalling pathway, this paper has highlighted an important functional link between proteolytic processing of cell-surface adhesion molecules and transcriptional activation in the nucleus.

Rachel Smallridge

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