SIGNALLING

The GEP100 pathway to invasion

Activation of the epidermal growth factor receptor (EGFR) can lead to tumour metastasis, but it is still not clear which signalling pathways downstream of EGFR mediate invasion. In *Nature Cell Biology*, Hisataka Sabe and colleagues now report that the ADP-ribosylation factor (ARF) guanine nucleotide-exchange factor (GEF) ARFGEP100 induces breast cancer invasion by specifically activating the GTPase ARF6 in response to EGF.

It has previously been shown that ARF6 is present at low levels in normal or non-invasive breast cancer cells, but it is highly expressed in invasive breast tumours and is crucial to the development of malignancy. Invasive MDA-MB-231 breast cancer cells express ten different ARFGEFs, so Sabe and colleagues set out to test their relative importance. RNA-mediated silencing of one of these, *ARFGEP100*, prevented the formation of invadopodia and blocked invasion in a Matrigel invasion assay; however, silencing the other GEFs had no effect. This suggested that ARFGEP100 specifically controls the invasive properties of breast tumour cells, possibly through the control of ARF6.

The authors confirmed this by showing that EGF-induced Matrigel invasion by MDA-MB-231 cells was blocked upon knockdown of either ARF6 or ARFGEP100, and activation of ARF6 was prevented in the absence of ARFGEP100. Furthermore, properties such as viability, adhesion or migration in response to surface-bound chemoattractants remained unaffected, showing that ARFGEP100 activates ARF6 downstream of EGFR specifically to promote invasion.

Sabe and colleagues went on to study the molecular mechanisms leading to ARF6 activation. Coimmunoprecipitation experiments showed that ARFGEP100 and ligand-activated EGFR interact in MDA-MB-231 cells. By ectopically expressing tagged versions of EGFR, ARFGEP100 and ARF6 in Cos-7 cells, they confirmed that the EGFR–ARFGEP100 interaction led to ARF6 activation. The authors used truncated versions of ARFGEP100 and mutated EGFRs to show that ARFGEP100 interacts with two of the six EGFR phosphotyrosine sites — Tyr 1068 and Tyr 1086 — through its pleckstrin homology domain.

The majority of malignant breast carcinoma cells co-express EGFR and ARFGEP100. When ARFGEP100 and ARF6 were simultaneously and ectopically expressed in the presence of EGF they were able to convert breast cancer cells from non-invasive to invasive. This suggests that the EGFR–ARFGEP100–ARF6 pathway might be activated in breast cancer cells that acquire a malignant phenotype. Finally, the authors showed that silencing ARFGEP100 in a mouse mammary cancer cell line led to fewer and smaller metastases when cells were injected in mammary fat pads, providing evidence that ARFGEP100 affects tumour malignancy *in vivo*.

Taken together, the results presented in this work indicate that EGF-stimulated ARFGEP100 and EGFR form a complex through the binding of the ARFGEP100 pleckstrin homology domain to tyrosine-phosphorylated EGFR, and that this activates ARF6 to promote metastasis. This signalling pathway, absent in normal cells and specifically present in tumour invasion, might constitute an ideal target for the development of therapeutic cancer drugs.

Kim Baumann
Editor
Cell Migration Gateway

ORIGINAL RESEARCH PAPER Morishige, M. *et al.* GEP100 links epidermal growth factor receptor signalling to ARF6 activation to induce breast cancer invasion. *Nature Cell Biol.* **10**, 85–92 (2008)