Ubiquitin-mediated regulation of RhoGTPase signalling: IAPs and HACE1 enter the fray

Mariam Orme, Katuscia Bianchi and Pascal Meier*

The Breakthrough Toby Robins Breast Cancer Research Centre, Institute of Cancer Research, Mary-Jean Mitchell Green Building, Chester Beatty Laboratories, London, UK

*Correspondence to: pmeier@icr.ac.uk

The EMBO Journal (2012) 31, 1–2. doi:10.1038/emboj.2011.452

Activation of members of the Rho-like family of guanosine triphosphatases (RhoGTPases) controls diverse physiological processes and is frequently found in cancer, contributing to tumour malignancy, cancer cell migration, invasion and metastasis. While the regulation of nucleotide binding to RhoGTPases is well understood, little is currently known regarding the molecular mechanisms through which RhoGTPase signalling is regulated by ubiquitylation. Two reports in this issue of *The EMBO Journal* and *Developmental Cell* now identify inhibitor of apoptosis (IAP) proteins and HACE1 as E3 ubiquitin (Ub)-protein ligases for Rac1 regulating Rac1 levels and activity.

Most members of the RhoGTPase protein family function as molecular switches that cycle between an inactive GDP-

bound form and an active GTP-bound state (Vega and Ridley, 2008). Binding of GTP to RhoGTPases, such as Rac1, triggers a conformational change that allows binding and activation of downstream effector proteins, through which Rac1 modulates actin assembly, actomyosin contractility, and microtubule formation. Activation of RhoGTPases, and hence their potential to interact with downstream signalling molecules, is influenced by a range of auxiliary proteins. Guanine-nucleotide exchange factors (GEFs) catalyse the exchange of GDP for GTP, thus activating the RhoGTPase. By contrast, GTPase-activating proteins (GAPs) promote the intrinsic GTPase activity, thereby accelerating the hydrolysis of GTP to GDP and returning RhoGTPases to their inactive configuration. RhoGTPases can also be kept inactive by association with

Figure 1 Schematic overview of Ub-mediated regulation of Rac1. The activity status of Rac1 is determined by its association with GAPs, GEFs and GDIs as well as through post-translational modifications. Ubiquitylation and degradation of Rac1 reportedly occurs in the nucleus and is mediated by IAPs and HACE1. Polyubiquitylation and degradation of Rac1 also requires calveolin1 and the nuclear import factor karyopherin- α 2. While XIAP, cIAP1 and HACE1 target activated Rac1 for proteasomal degradation, inactive, GDP-bound Rac1 can only be degraded by IAPs but not HACE1. Inhibition of IAPs and HACE1 stabilises Rac1 and causes morphological changes that influence cell migration and protects cells from intoxication of CNF1, a toxin produced by uropathogenic *E. coli* that targets Rac1, and other RhoGTPases, for proteasomal degradation.

cytosolic chaperone proteins known as guanine-nucleotide dissociation inhibitors (GDIs), which block the action of GEFs, and extract GDP-bound RhoGTPases from membranes where they may otherwise become activated inappropriately. In addition to modulation by GEFs, GAPs and GDIs, RhoGTPases are subject to regulation via post-translational modification. For example, addition of lipidic groups to their C-termini enhance their interaction with membranes, while conjugation of Ub to RhoGTPases targets them for inactivation via proteasomal degradation (Nethe and Hordijk, 2010).

By interrogating the role of IAP proteins in health and disease, Krishnaraj Rajalingam and colleagues noticed that silencing of the E3s XIAP and cIAP1 led to Rac1 stabilisation, elongated morphology and enhanced migration (Oberoi *et al*, 2012) (see Figure 1). Consistent with this notion, they demonstrate that XIAP and cIAP1 directly bind to Rac1 in a nucleotide-independent manner *in vitro* and *in vivo*, and that both these IAPs can conjugate polyubiquitin chains to Rac1, targeting it for proteasomal degradation. They further demonstrate that these IAPs are also required for Rac1 degradation following expression of RhoGDI, and exposure to cytotoxic necrosis factor 1 (CNSF1)—a toxin that is produced by uropathogenic *Escherichia coli* (UPEC) and targets members of the RhoGTPases, including Rac1, for proteasomal degradation. In the absence of XIAP and cIAPs, however, RhoGDI- and CNSF1-mediated depletion of Rac1 is prevented. IAPs appear not to be the only E3s that target Rac1 for proteasomal degradation following CNSF1 intoxication. Elegant work by Emmanuel Lemichez and colleagues demonstrate that the HECT-type E3 HACE1 is required for CNSF1-mediated depletion of Rac1 (Torrino *et al*, 2011) (see Figure 1). They further demonstrate that in the absence of HACE1, UPEC fail to efficiently invade endothelial cell monolayers, suggesting that HACE1 plays a major role in host defence against pathogens. The relative contributions of IAPs and HACE1 to Rac1 turnover remain to be clarified, but it is interesting to note that while HACE interacts specifically with active Rac1, IAPs seem to be indiscriminate about the activation state of Rac1.

IAPs also regulate migration of certain neuronal progenitor cells of the developing cerebellum of zebrafish. Normally,

cells migrate from the upper rhombic lip region of the cerebellum to their final destination in several of the tegmental hindbrain nuclei and in the cerebellar cortex (Tahirovic *et al*, 2010). Strikingly, either expression of a dominant-negative form of Rac1, or overexpression of XIAP, in the upper rhombic lip cells impaired directional migration of these cells to their final destination. The phenotype caused by XIAP overexpression was partially rescued by co-expression of wild-type Rac1, indicating that XIAP exerts its effects through Rac1.

The ability of mammalian IAPs to regulate migration via controlling Rac1 appears to be evolutionarily conserved, as the *Drosophila* IAP1 (DIAP1) has previously been reported to bind to Rac and regulate collective migration of a group of cells in the developing egg chamber (Geisbrecht and Montell, 2004). However, while the report by Oberoi *et al*, and previous data from the same group (Dogan *et al*, 2008), suggest that IAPs inhibit cell migration, the data from *Drosophila* (Geisbrecht and Montell, 2004), and other reports from mammalian systems (Mehrotra *et al*, 2010; Liu *et al*, 2011; Lopez *et al*, 2011), indicate that IAPs actually promote migration. Clarification of this discrepancy will be extremely important, not least because pharmacological inhibitors of IAPs (dubbed Smac mimetics) are undergoing clinical trials for the treatment of cancer (Dynek and Vucic, 2010). While Smac mimetics were developed to inactivate IAPs and induce tumour cell death, the study by Rajalingam and colleagues now suggests that treatment with IAP inhibitors may promote migration of surviving tumour cells and thus increase the risk of metastasis. Unravelling how IAPs regulate cell migration and invasion will be critically important, as metastasis represents the major clinical problem in cancer.

Acknowledgements

We acknowledge support from CRUK and MRC as well as NHS funding to the NIHR Biomedical Research Centre.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- Dogan T, Harms GS, Hekman M, Karreman C, Oberoi TK, Alnemri ES, Rapp UR, Rajalingam K (2008) X-linked and cellular IAPs modulate the stability of C-RAF kinase and cell motility. *Nat Cell Biol* **10**: 1447–1455
- Dynek JN, Vucic D (2010) Antagonists of IAP proteins as cancer therapeutics. *Cancer Lett* (advance online publication, 2 August 2010)
- Geisbrecht ER, Montell DJ (2004) A role for *Drosophila* IAP1-mediated caspase inhibition in Rac-dependent cell migration. *Cell* **118**: 111–125
- Liu J, Zhang D, Luo W, Yu Y, Yu J, Li J, Zhang X, Zhang B, Chen J, Wu XR, Rosas-Acosta G, Huang C (2011) X-linked inhibitor of apoptosis protein (XIAP) mediates cancer cell motility via Rho GDP dissociation inhibitor (RhoGDI)-dependent regulation of the cytoskeleton. *J Biol Chem* **286**: 15630–15640
- Lopez J, John SW, Tenev T, Tautureau GJP, Hinds MG, Francalanci F, Wilson R, Broemer M, Santoro MM, Day CL, Meier P (2011) CARD-mediated autoinhibition of cIAP1's E3 ligase activity suppresses cell proliferation and migration. *Mol Cell* **42**: 569–583
- Mehrotra S, Languino LR, Raskett CM, Mercurio AM, Dohi T, Altieri DC (2010) IAP regulation of metastasis. *Cancer Cell* **17**: 53–64
- Nethe M, Hordijk PL (2010) The role of ubiquitylation and degradation in RhoGTPase signalling. *J Cell Sci* **123**: 4011–4018
- Oberoi TK, Dogan T, Hocking JC, Scholz R-P, Mooz J, Anderson CL, Karreman C, Meyer zu Heringdorf D, Schmidt G, Ruonala M, Namikawa K, Harms GS, Carpy A, Macek B, Köster RW, Rajalingam K (2012) IAPs regulate the plasticity of cell migration by directly targeting Rac1 for degradation. *EMBO J* **31**: 14–28
- Tahirovic S, Hellal F, Neukirchen D, Hindges R, Garvalov BK, Flynn KC, Stradal TE, Chrostek-Grashoff A, Brakebusch C, Bradke F (2010) Rac1 regulates neuronal polarization through the WAVE complex. *J Neurosci* **30**: 6930–6943
- Torrino S, Visvikis O, Doye A, Boyer L, Stefani C, Munro P, Bertoglio J, Gacon G, Mettouchi A, Lemichez E (2011) The E3 ubiquitin-ligase HACE1 catalyzes the ubiquitylation of active Rac1. *Dev Cell* **21**: 959–965
- Vega FM, Ridley AJ (2008) Rho GTPases in cancer cell biology. *FEBS Lett* **582**: 2093–2101