It's not over until the FAT lady sings

Marco J Herold^{1,2} & Andreas Strasser^{1,2}

The death receptors FAS, TRAIL-Rs and TNFR1 play critical roles in programmed cell death, particularly in the immune system. Upon ligation of death receptors, caspase-8 is activated within the so-called 'Death Induced Signalling Complex' (DISC) but the mechanisms that mediate and modulate the activation of caspase-8 are still not fully understood. This is an important issue because caspase-8 is essential for apoptosis induced by death receptors. In this issue of *The EMBO Journal*, Kranz and Boutros (2014) describe their findings from a whole genome siRNA screen for the identification of novel regulators of death receptor induced apoptosis signalling. They identified the atypical cadherin FAT1 as a negative regulator of TRAIL-R-mediated caspase-8 activation and consequent induction of apoptosis, although it had no impact on NF- κ B activation. The authors also show that FAT1 depletion substantially increased TRAIL-induced killing of glioblastoma-derived cell lines, suggesting a potential novel approach for treatment of this highly aggressive cancer.

See also: D Kranz & M Boutros (January 2014)

The death receptor-induced apoptotic pathway is a mechanism to eliminate unwanted and/or potentially dangerous (e.g. pathogen infected) cells (Strasser *et al*, 2009). Ligand-induced aggregation with consequent activation of pre-assembled trimers of death receptors (e.g. FAS, TNFR1 or TRAIL-Rs) leads to the formation of a so-called 'death-inducing signalling complex' (DISC) (Kischkel *et al*, 1995) at the inside of the plasma membrane. This DISC is comprised of the death receptor, the adaptor protein FADD (and for some but not all death receptors also TRADD), caspase-8

and its inhibitor c-FLIP. This leads to the activation of caspase-8, an aspartate-specific cysteine protease, which triggers apoptosis by proteolytic activation of the effector caspases (caspase-3 and -7) and the pro-apoptotic BH3-only protein BID, the latter to further amplify the caspase cascade by engaging the BCL-2-regulated apoptotic pathway (Jost *et al*, 2009).

Mechanistically, activation of caspase-8 was initially believed to be explained by the 'induced proximity model' (Salvesen & Dixit, 1999), whereby two caspase-8 molecules are brought together in the DISC via FADD, which connects the death receptors via homotypic death domain-death domain (DD) and death effector domain-death effector domain (DED) interactions with the caspase-8 molecules (Fig 1). It is, however, now established that the mere proximity of two caspase-8 molecules with the resulting conformational change (Hughes *et al*, 2009) is not sufficient for full enzymatic activation. This form of caspase-8 activation at the plasma membrane, now known to involve a complex with c-FLIP (Oberst *et al*, 2011), is sufficient to prevent the embryonic lethality (~E9.5) caused by complete loss of caspase-8 (Kang *et al*, 2004), which was revealed to be due to RIPK3- and MLKL-mediated necroptosis (Kaiser *et al*, 2011; Oberst *et al*, 2011; Murphy *et al*, 2013). For induction of apoptosis, caspase-8 must cleave itself at specific sites to allow formation of a (p20₂p10₂) hetero-tetrameric protease that must be released into the cytosol to gain access to its critical apoptotic substrates, caspase-3, -7 and BID (Kang *et al*, 2004). Moreover, the recruitment of caspase-8 into the DISC does not follow a 1:1 stoichiometry with the FADD molecules, but many more caspase-8 molecules (than FADD molecules) assemble as chains at the underside of the plasma

membrane (Schleich *et al*, 2012; Dickens *et al*, 2012; Fig 1). Cullin3-mediated ubiquitination and subsequent p62 recruitment was reported to stabilise these large caspase-8 containing complexes (Jin *et al*, 2009).

Functional genomics using RNAi is a powerful approach for the identification of novel or previously unrecognised components of signalling pathways of interest. In this issue of *The EMBO Journal*, Kranz and Boutros (2014) report on a whole mouse genome siRNA screen to identify regulators that when knocked down enhance TRAIL-induced apoptosis (in a so-called 'synthetic lethal' screen). Surprisingly they identified the atypical cadherin FAT1 as a negative regulator of caspase-8, although this protein has never been associated with death receptor signalling before. FAT1 belongs to a family of cadherins that was originally identified in *Drosophila* and can be subdivided into the FAT and FAT-like members. Mammalian FAT4 shows the largest extent of homology to *Drosophila* FAT, whereas mammalian FAT1, FAT2 and FAT3 are more closely related to *Drosophila* FAT-like. The *Drosophila* gene *Fat* encodes a tumour suppressor that restricts cellular proliferation during development. In human glioblastoma, head-and-neck as well as colorectal cancers, somatically acquired mutations or deletion of FAT1 were found to be associated with enhanced tumour growth, and this was attributed to increased WNT signalling. Interestingly, Kranz and Boutros (2014) showed that RNAi-mediated knock-down of FAT1 sensitises primary glioblastoma cells to TRAIL-induced apoptosis. They used the novel and very exciting CRISPR/Cas9 genome editing technology to generate FAT1-deficient glioblastoma lines to confirm this observation. These results indicate that glioblastomas harbouring inactivating mutations or deletions of FAT1

1 The Walter and Eliza Hall Institute, Melbourne, Australia

2 Department of Medical Biology, University of Melbourne, Melbourne, Australia. E-mail: herold@wehi.edu.au

DOI 10.1002/embj.201387542

Figure 1. A model to show the proposed mechanism by which FAT1 modulates death receptor induced apoptosis signalling by impairing the activation of caspase-8 within high molecular weight complexes at the underside of the plasma membrane.

By inhibiting caspase-8 activation, FAT1 may increase the overall output of NF- κ B signaling from activated death receptors (by keeping cells alive for longer). Since loss or inhibition of caspase-8 can also promote induction of death receptor induced necroptosis, FAT1 may also play a role in this regulatory switch.

might respond to low-dose TRAIL, a treatment that would be expected to have little impact on healthy tissues, in part due to their expression of FAT1.

How does FAT1 inhibit caspase-8 activity? The authors showed that upon TRAIL-R stimulation FAT1 interacts with caspase-8 to prevent the formation of the high-molecular-weight complexes (HMWC) that contain active caspase-8 (Fig 1). Accordingly, FAT1 knock-down caused increased accumulation of caspase-8 molecules in HMWC at the plasma membrane, probably representing the recently described activating assembly chains of caspase-8 (Dickens *et al*, 2012; Schleich *et al*, 2012). It is therefore tempting to hypothesise that FAT1 somehow interrupts the assembly of caspase-8 chains at the DISC, thereby dampening activation of the downstream effector caspases. However, death receptors do not only trigger apoptotic cell death but can under certain conditions also activate NF- κ B signalling, which promotes cell survival, proliferation, migration and inflammatory cytokine production. Interestingly, FAT1 knock-down did not appear to affect TNF α -induced NF- κ B signalling (reflected by no impact on cytokine

production). Nevertheless, it remains possible that FAT1 modulation of caspase-8 activity could affect the balance between pro-apoptotic versus NF- κ B signalling, triggered by death receptors, simply by keeping cells alive for a longer time, thereby facilitating increased overall production of inflammatory cytokines.

In conclusion, the paper by Kranz and Boutros (2014) in this issue of *The EMBO Journal* presents FAT1 as a novel and unexpected regulator of caspase-8 activation and hence the death receptor apoptotic pathway. This work nicely exemplifies the power of siRNA library screening for the discovery of novel regulators and the utility of the CRISPR/Cas9 genome editing technology for functional validation. The findings raise several exciting questions: How does FAT1 regulate recruitment and activation of caspase-8 within the DISC? What is the impact of FAT1 loss in non-cancerous cells? Are different FAT and FAT-like family members involved in different apoptotic processes or do they have no role in cell death? Finally, it will be interesting to examine whether FAT1 also regulates caspase-8 activity in other cell death processes,

particularly necroptosis, which only occurs when caspase-8 is deleted or its enzymatic activity blocked (Murphy *et al*, 2013).

Acknowledgments

Work in our laboratories is supported by the National Health and Medical Research Council, Australia (Program Grant 1016701 and Fellowship 1020363) and the Leukemia & Lymphoma Society (Specialized Center of Research 7001-13) and was made possible through Victorian State Government Operational Infrastructure Support and Australian Government NHMRC IRISS.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- Dickens LS, Boyd RS, Jukes-Jones R, Hughes MA, Robinson GL, Fairall L, Schwabe JW, Cain K, Macfarlane M (2012) A death effector domain chain DISC model reveals a crucial role for caspase-8 chain assembly in mediating apoptotic cell death. *Mol Cell* 47: 291–305
- Hughes MA, Harper N, Butterworth M, Cain K, Cohen GM, MacFarlane M (2009) Reconstitution of the death-inducing signaling complex reveals a substrate switch that determines CD95-mediated death or survival. *Mol Cell* 35: 265–279
- Jin Z, Li Y, Pitti R, Lawrence D, Pham VC, Lill JR, Ashkenazi A (2009) Cullin3-based polyubiquitination and p62-dependent aggregation of caspase-8 mediate extrinsic apoptosis signaling. *Cell* 137: 721–735
- Jost PJ, Grabow S, Gray D, McKenzie MD, Nachbur U, Huang DC, Bouillet P, Thomas HE, Borner C, Silke J, Strasser A, Kaufmann T (2009) XIAP discriminates between type I and type II FAS-induced apoptosis. *Nature* 460: 1035–1039
- Kaiser WJ, Upton JW, Long AB, Livingston-Rosanoff D, Daley-Bauer LP, Hakem R, Caspari T, Mocarski ES (2011) RIP3 mediates the embryonic lethality of caspase-8-deficient mice. *Nature* 471: 368–372
- Kang TB, Ben-Moshe T, Varfolomeev EE, Pewzner-Jung Y, Yogev N, Jurewicz A, Waisman A, Brenner O, Haffner R, Gustafsson E, Ramakrishnan P, Lapidot T, Wallach D (2004) Caspase-8 serves both apoptotic and nonapoptotic roles. *J Immunol* 173: 2976–2984
- Kischkel FC, Hellbardt S, Behrmann I, Germer M, Pawlita M, Krammer PH, Peter ME (1995) Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor. *EMBO J* 14: 5579–5588

- Kranz D, Boutros M (2014) A synthetic lethal screen identifies FAT1 as an antagonist of caspase-8 in extrinsic apoptosis. *EMBO J* 33: 181–197
- Murphy JM, Czabotar PE, Hildebrand JM, Lucet IS, Zhang JG, Alvarez-Diaz S, Lewis R, Lalaoui N, Metcalf D, Webb AI, Young SN, Varghese LN, Tannahill GM, Hatchell EC, Majewski IJ, Okamoto T, Dobson RC, Hilton DJ, Babon JJ, Nicola NA *et al* (2013) The Pseudokinase MLKL Mediates Necroptosis via a Molecular Switch Mechanism. *Immunity* 39: 443–453
- Oberst A, Dillon CP, Weinlich R, McCormick LL, Fitzgerald P, Pop C, Hakem R, Salvesen GS, Green DR (2011) Catalytic activity of the caspase-8-FLIP(L) complex inhibits RIPK3-dependent necrosis. *Nature* 471: 363–367
- Salvesen GS, Dixit VM (1999) Caspase activation: the induced-proximity model. *Proc Natl Acad Sci USA* 96: 10964–10967
- Schleich K, Warnken U, Fricker N, Oztürk S, Richter P, Kammerer K, Schnölzer M, Krammer PH, Lavrik IN (2012) Stoichiometry of the CD95 death-inducing signaling complex: experimental and modeling evidence for a death effector domain chain model. *Mol Cell* 47: 306–319
- Strasser A, Jost PJ, Nagata S (2009) The many roles of FAS receptor signaling in the immune system. *Immunity* 30: 180–192