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The roles of FADD in extrinsic apoptosis and necroptosis

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Fas-associated protein with death domain (FADD), an adaptor that bridges death receptor signaling to the caspase cascade, is indispensible for the induction of extrinsic apoptotic cell death. Interest in the non-apoptotic function of FADD has greatly increased due to evidence that FADD-deficient mice or dominant-negative FADD transgenic mice result in embryonic lethality and an immune defect without showing apoptotic features. Numerous studies have suggested that FADD regulates cell cycle progression, proliferation, and autophagy, affecting these phenomena. Recently, programmed necrosis, also called necroptosis, was shown to be a key mechanism that induces embryonic lethality and an immune defect. Supporting these findings, FADD was shown to be involved in various necroptosis models. In this review, we summarize the mechanism of extrinsic apoptosis and necroptosis, and discuss the *in vivo* and *in vitro* roles of FADD in necroptosis induced by various stimuli. [BMB Reports 2012; 45(9): 496-508]

INTRODUCTION

Fas-associated protein with death domain (FADD) is a critical adaptor protein for death receptor (DR)-mediated apoptosis. FADD is composed of two domains called the death domain (DD) and death effector domain (DED). The DD of FADD binds to the DD of the death receptor and FADD recruits procaspase-8 through the DED-DED interaction, forming a death-inducing signaling complex (DISC), where procaspase-8 is activated by self-cleavage. Active caspase-8 cleaves downstream effector caspases such as caspase-3, -6, and -7, inducing apoptosis.

Interestingly, FADD deficiency results in embryonic lethality, displaying a defect in immune homeostasis and immune cell proliferation despite the defect in inducing apoptosis. In addition, FADD is also implicated in non-apoptotic functions such as cell cycle progression, proliferation, autophagy, inflammation and innate immunity (1, 2). Particularly, FADD phosphorylation at Ser194 (pFADD) by several kinases is associated with its nuclear localization and cell cycle regulation (3-8). Although FADD and pFADD are often overexpressed in various tumors, their functions in cancer development or chemotherapy- sensitivity are still controversial (9-14).

Recent strong evidence from *in vivo* mice studies suggested negative roles of FADD in RIP1- and RIP3-dependent necroptosis (15-18). DR-mediated caspase-8 activation requires FADD, and leads to the cleavage of RIP1, RIP3, and CYLD, preventing necroptosis (19-22). Thus, FADD deficiency is thought to inhibit caspase-8 and subsequent apoptosis, but activate necroptosis. Since necroptosis can be initiated by various stimuli in a variety of cell types independently of DRs, the exact mechanisms and functions of FADD require further investigation. This review focuses on the recent discoveries about the roles of FADD in apoptosis and necroptosis in various models, and we refer the reader to two comprehensive reviews of the diverse functions of FADD (1, 2).

EXTRINSIC APOPTOSIS

Molecular mechanism of extrinsic apoptosis

Extrinsic apoptosis, which is triggered by the extracellular signals that activate the death receptor family, is distinguished from intrinsic apoptosis, which is induced by intracellular signals such as DNA damage, oxidative stress, and nutrient deprivation (23). Extrinsic apoptosis is initiated by the binding of specific ligands such as tumor necrosis factor α (TNFα), Fas ligand (FasL), and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) to their corresponding receptors called 'death receptors' (DRs) (24). DR is a member of the TNF receptor superfamily and specifically contains a conserved cytosolic death domain (DD) (25). The eight kinds of DRs have different amino acid sequences that determine ligand specificity, and they can be divided into two groups according to the cytosolic adaptor protein that makes a distinct complex (24, 26, 27).

The first group includes CD95/Fas, DR4/TRAIL-R1, and DR5/TRAIL-R2, all of which recruit death-inducing signaling complex (DISC) composed of FADD and procaspase-8 (28). Fas and DR4/5 are activated by the ligation of the specific ligands FasL and TRAIL, respectively, and bind to the DD of FADD, a pivotal adaptor protein, through the DD domain. Then, DED of FADD binds to DED of procaspase-8 and -10 to construct the DISC. The

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DISC facilitates auto-proteolytic cleavage of procaspase-8 and -10, which confers their enzymatic activity and release (2). Activated caspase-8 and -10 lead to proteolytic stimulation of downstream effector caspase-3, -6, and -7, which can cleave intracellular substrates such as lamin A, poly (ADP-ribose) polymerase (PARP), and inhibitor of caspase-activated DNase (ICAD) to induce apoptotic circumstances including cell shrinkage, nuclear fragmentation, apoptotic DNA fragmentation, and ultimately, cell death (24). The series of events described above is sufficient to induce apoptotic cell death in certain cell types called type I cells, such as lymphocytes and thymocytes, but is insufficient in type II cells, including hepatocytes and pancreatic β cells, because of the relatively low levels of DISC in spite of comparable levels of the DISC components. (29-32). Type II cells require a mitochondria-dependent pathway to amplify a DR-mediated apoptotic signaling. Activated caspase-8 cleaves pro-apoptotic protein BID as well as effector caspases to generate truncated BID (tBID), which binds to pro-apoptotic proteins BAX and BAK, resulting in the leakage of the mitochondrial membrane and the release of cytochrome c and SMAC/DIABLO (33, 34). Released cytochrome c forms an apoptosome with procaspase-9 and Apaf-1 to cleave and activate caspase-9, which in turn stimulates caspase-3, -6, and -7 (24). SMAC/DIABLO, meanwhile, facilitates apoptosis by suppressing inhibitors of apoptosis proteins (IAPs) (35, 36).

The second group of DRs including TNFR1, DR3, DR6, and EDAR recruit TRADD for an adaptor protein that links DRs to TNF receptor-associated factors 2,5 (TRAF2,5), receptor-interacting protein kinase (RIP1 or RIPK1), and cellular inhibitor of apoptosis (cIAPs), forming a signaling complex called 'complex I' (24, 37). Upon the ligation of DR with their specific ligands, complex I is assembled close to the plasma membrane to stimulate mitogen-activated protein kinase/c-Jun N-terminal kinases (MAPK/JNK) involved in cell survival, proliferation or apoptosis (38-40). Complex I also stimulates nuclear factor kappa (NF-κB) pathway, facilitating cell survival and inflammatory signal (38). Complex I-mediated NF-κB stimulation is caused by cIAP-induced K63-linked polyubiquitination of RIP1 and linear ubiquitin chain assembly complex (LUBAC)-mediated linear ubiquitination of RIP1, providing a scaffold for the recruitment of TGF-beta-activated kinase 1 (TAK1) binding protein 2 and 3 (TAB2 and 3), which finally activates TAK1 (41-43). IKKγ/NEMO also binds to the polyubiquitin chain of RIP1, bringing the whole IKK complex. Then, active TAK1 phosphorylates and stimulates IKKβ, resulting in the phosphorylation and subsequent degradation of IκB, which sequesters NFκB in the cytosol (42). Subsequently, unconstrained NFκB enters the nucleus to turn on the transcription of its target gene encoding anti-apoptotic, pro-survival, and inflammatory factors. The second group of DRs can also form a cytosolic complex II. RIP1 deubiquitinating enzymes such as cylindromatosis (CYLD) remove K63-linked polyubiquitination, leading to internalization of receptor complex (38, 44). It is unclear whether the deubiquitination function of A20, ubiquitin-specific protease 21 (USP21) and cezanne (OTUB7B) are also able to induce complex II formation (45-48). The conformation changes of complex I after receptor internalization result in two kinds of cytosolic complex II (i.e., TRADD-dependent complex IIA and RIP1-dependent complex IIB), both of which can initiate apoptosis (38, 44). TRADD recruits FADD and caspase-8, forming complex IIA, where caspase-8 is activated and apoptosis is initiated (49, 50). Complex IIB is composed of RIP1 and FADD-caspase-8 and is negatively regulated by cIAPs since polyubiquitinated RIP1 cannot be incorporated into complex IIB. Therefore, IAP antagonist, including smac mimetics that induce the proteasomal degradation of IAPs, can promote complex II formation and subsequent apoptosis (51, 52).

Regulation of extrinsic apoptosis

Several regulatory machineries are involved in the DR-mediated extrinsic apoptosis pathway. Cellular FLICE-like inhibitory proteins (cFLIPs) are crucial regulators of DR signaling (See ref. 53 for Review). All of the cFLIP isoforms, including cFLIP long ($cFLIP_L$), cFLIP short ($cFLIP_S$), and cFLIP raji ($cFLIP_R$), contain two DED domains and bind to FADD via DED-DED interaction (53). All cFLIPs prevent DISC formation and consequent apoptosis by competing with caspase-8 for binding to FADD (54). The role of $cFLIP_L$ on apoptosis, however, is still controversial. $cFLIP_L$ also contains a caspase-8-like domain and forms a heterodimer with caspase-8, resulting in partial caspase-8 autoprocessing that is sufficient to generate the p43/41 and the p12 fragments (55, 56). The levels of cFLIP are regulated by numerous pathways. For instance, JNK activated by TNFα can phosphorylate and stimulate the E3 ubiquitin ligase, Itch, inducing polyubiquitination and proteasomal degradation of cFLIP (57). Moreover, the phosphatidylinositol 3-kinase/Akt pathway can upregulate cFLIP expression (58).

Various post-translational modifications (PTMs) are also implicated in the regulation of DR-mediated extrinsic apoptosis. DRs can be directly modified by several PTMs (59, 60). For example, palmitoylation of Fas/CD95 facilitates the formation of high molecular weight DISC, which includes FADD and caspase-8, resulting in caspase-8 cleavage and apoptotic cell death (60). O-glycosylation of TRAIL-R1/2 results in improved receptor clustering and subsequent DISC formation, causing better sensitivity to TRAIL (61). Furthermore, cFLIP is also regulated by various PTMs including nitrosylation, ubiquitylation, and phosphorylation (62-66). For example, phosphorylation of cFLIP isoform by protein kinase C (PKC) does not affect its interaction with the DISC. On the other hand, phosphorylation of $cFLIP_S$ induces stabilization of $cFLIP_S$ by reducing polyubiquitination, enhancing its anti-apoptotic function (62). S-receptor kinase (Srk) phosphorylates caspase-8, prevents procaspase-8 cleavage, and impairs DRs Fas-mediated apoptosis (67). Caspase-8/10-associated RING proteins (CARPs) suppress caspase-8 and -10 via ubiquitin-mediated degradation. Therefore, down-regulation of CARPs enhances DR-mediated apoptosis in human lung cancer cells (68). CARP2 also mediates K48-linked polyubiquitination and degra-