Death effecter domain for the assembly of death-inducing signaling complex

Jin Kuk Yang

Abstract Death-inducing signaling complex (DISC) is a platform for the activation of initiator caspase in extrinsic apoptosis. Assembly of DISC is accomplished by two different types of homotypic interaction: one is between death domains (DDs) of a death receptor and FADD, and the other is between death effecter domains (DEDs) of FADD, procaspase-8/-10 and cFLIP. Recent biochemical investigations on the stoichiometry of DISC have revealed that single-DED-containing FADD exists in DISC in a substantially lower abundance than the sum of tandem-DEDs-containing components that are procaspase-8 and cFLIP. In addition, the homology models of the tandem DEDs in procaspase-8 and cFLIP show that two different interaction faces, H1–H4 face and H2–H5 face, are exposed for possible inter-molecular DED–DED interactions. These recent findings led to a proposal of the DED chain model for the interactions between FADD, procaspase-8 and cFLIP in DISC. This emerging view provides new insights on the topology of DED–DED network in DISC and furthermore on how procaspase-8 and cFLIP cluster for dimerization and proteolytic activation.

Keywords Death effecter domain · Death-inducing signaling complex · Apoptosis · Inflammation

Introduction

Death effecter domain (DED) is a member of death domain (DD) superfamily of which the other members are DD,

caspase recruitment domain (CARD) and pyrin domain (PYD) [1]. The common structural feature of the four subfamily domains is a hexa-helical bundle fold with a little deviation for each case [2–5]. The four domains are found in a large number of proteins mainly involved in cell death and inflammation signaling pathways, and thereby play essential roles as protein–protein interaction modules. Despite the fold similarity, the four domains exhibit very strict specificity of homotypic interactions so that DED, for example, interacts with only with DED, not with any DD, CARD or PYD [1]. These homotypic interactions of the four subfamily domains are essential for the assembly of the macromolecular complex where initiator caspases are activated through the proteolysis at their specific positions. For example, intrinsic apoptosis requires the formation of apoptosome consisting of cytochrome C, Apaf-1 and pro-caspase-9 [6, 7.] The CARD–CARD interaction between Apaf-1 and procaspase-9 is crucial for the assembly of the apoptosome at which the caspase-9 is activated [8]. On the other hand, the death-inducing signaling complex (DISC) for extrinsic apoptosis is composed of a membrane receptor Fas, cytosolic adaptor FADD, and procaspase-8/-10/cFLIP. For the assembly of the DISC, two different homotypic interactions of DD superfamily members are required: first, DD–DD interaction between Fas and Fadd, and second, DED–DED interaction between Fadd, procaspase-8/-10, and cFLIP [9–12]. Fas has a DD in its cytosolic part and procaspase-8/-10/cFLIP has two DEDs in tandem in its N-terminal part. FADD contains both DD and DED, and thereby links DD-containing Fas and DED-containing procaspase-8/-10/cFLIP for the assembly of DISC through the two different types of homotypic interactions that are DD–DD and DED–DED, respectivly [1]. The overall structure of the apopsosome and the stoichiometry of the component proteins are well defined mainly by electron

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microscopy and X-ray crystallography studies [13, 14]. However, the overall picture for the DISC assembly is still largely unknown since its structural studies have been limited by its structural complexity arising from the multitude of DD–DD and DED–DED interactions and additionally from the linkage of these two types of interaction networks by FADD. Instead of the whole image of DISC, the crystal structures of dissected DD–DD complex was successfully determined [15, 16]. Regarding the DED–DED interaction in DISC, no experimentally determined structure is currently available. Instead, the crystal structures of tandem DEDs in two viral homologs of cFLIP were determined to reveal the structural details of intramolecular DED–DED interactions [17, 18], which provide the analogical insight for the inter-molecular DED–DED interactions in DISC between Fadd, procaspase-8/-10 and cFLIP. In addition, several biochemical analyses on the stoichiometry of DISC components have been also reported very recently [19–21]. From the synthesis of all these recent studies, an advanced structural image on DISC assembly has been emerging. In this review, the current view on the structural mechanism for the DISC assembly will be discussed with a focus on DED–DED interactions.

Death effecter domain proteins in DISC

Death receptor (DR) family, a subset of the tumor necrosis factor (TNF) superfamily, comprises Fas (also called CD95 and APO-1), TRAIL-R1 (DR4), -R2 (DR5) and TNF-R1 as well studied members [1]. An interesting aspect in DR signaling is that DRs not only trigger cell death but also cell proliferation depending on the types of cell and receptor, and biological circumstances [22]. Binding of the corresponding ligand to each DR (FasL for Fas, TRAIL for TRAIL-R1/-R2, and TNF to TNF-R1) leads to the formation of DISC consisting of oligomerized DR and multiple copies of Fadd, procaspase-8 and/or procaspase-10, and cFLIP. Interactions between DISC component proteins are based on two different kinds of homotypic interactions of DD and DED. For DD–DD interaction between DR and Fadd, a theoretical model of 3:3 binding ratio for DR and Fadd was initially expected mainly based on the notion that TNF superfamily receptors usually trimerize [23, 24]. However, this expectation soon confronted the revelation of the crystal structures showing that Fas DD and Fadd DD interacts 4:4 or 5:5 [15, 16], even though there is still ongoing debate on which ratio among the two possibilities represents the physiological situation. In case of DED–DED interactions in DISC, there has been no reported structural observation on any part of it yet. Instead, a glimpse on the intra-molecular interaction in tandem DEDs of viral homologs of cFLIP has been possible by X-ray crystallography [17, 18], which provides analogical

information on the structural aspects of inter-molecular DED–DED interactions in DISC.

DED proteins in DISC are FADD, procaspase-8, -10 and cFLIP (Fig. 1). FADD contains both DED and DD, and is recruited to a DR through DD–DD interaction [9, 10]. The second layer of DISC assembly is brought about by DED of FADD which recruits procasepase-8 or -10 through DED–DED interaction [11, 12]. Once clustered in DISC, the procaspase molecules are situated in close distance to each other, which leads to the dimerization of their C-terminal protease domains and partial activation of protease function. Then autoproteolysis occurs, in case of procaspase-8, at D216, D374 and D384 to generate the fully active dimer of p18-p10 caspase-8 which is released out of DISC (Fig. 1) [25]. Procaspase-10 is activated in the same manner except that the active protease is p23-p12 caspase-10 is produced from the autoproteolysis at D219 and D415 [26]. The procaspase-8 or -10 has two DEDs in tandem in its N-terminal part while FADD has one DED, which forecasts rather more complicated DED–DED interaction profile in DISC than simple one-to-one interaction between FADD and procaspase-8 or -10 (Fig. 1). In addition to FADD and procaspase-8/-10, FLIP is another component of DISC and is better called as cellular FLIP or cFLIP contrasting to its viral homolog, viral FLIP or vFLIP [22, 27]. Three isoforms of cFLIP has been most studied so far: one long isoform, cFLIP$_L$, and two short isoforms, cFLIP$_S$ and cFLIP$_R$ (Fig. 1). cFLIP$_L$ has a similar domain organization as procaspase-8/-10, possessing N-terminal tandem DEDs and C-terminal protease-like domain lacking a protease activity. Two short isoforms, cFLIP$_S$ and cFLIP$_R$, are commonly composed of only tandem DEDs without the protease-like domain. cFLIP$_L$ can modulate apoptosis in two opposite directions depending on its expression level. When abundant, it inhibits the activation of caspase-8 and thereby blocks the apoptosis initiation. In contrast, if its level is low as ∼1 % of procaspase-8, then cFLIP$_L$ promotes procaspase-8 activation [22, 28]. It is notable that cFLIP can be cleaved at D198 by procaspase-8 and at D376 by the activated caspase-8 to generate p22-cFLIP and p43-cFLIP respectively. These cleaved fragments has been shown to function mainly in NF-κB signaling for the survival and proliferation of lymphocytes [29, 30].

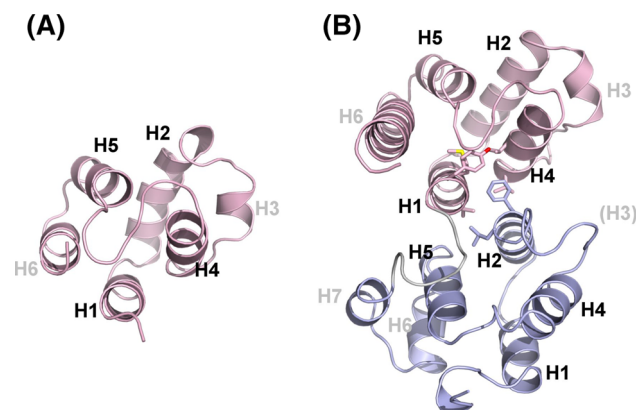Structural features of death effecter domain

DEDs of DISC component proteins (FADD, procaspase-8/-10 and cFLIP), when over-expressed, tend to aggregate, which have hampered their structural studies in vitro. FADD DED shows improved solution behavior with F25Y mutation which led to the first NMR structure determination for DED [3]. The NMR structure of FADD DED^{F25Y} showed a hexa-helical bundle structure which was later

Fig. 1 Domain organization of major DED-containing proteins in DISC. Proteolytic cleavage sites are indicated by *arrows* with the residue type and number. DED of FADD and C-DEDs in tandem-DEDs-containing proteins are colored same in *red* to indicate their sharing of canonical hexa-helical fold of DD superfamily. On the other hand, N-DEDs are in contrasting *blue color* to represent its structural deviation from the canonical fold. The *shaded* region in cFLIP$_R$ represents its unique 11-residue-long sequence which is contained in the other two isoforms, cFLIP$_L$ and cFLIP$_S$. For simplicity, among various vFLIPs, presented here are only two vFLIPs of which crystal structures were reported
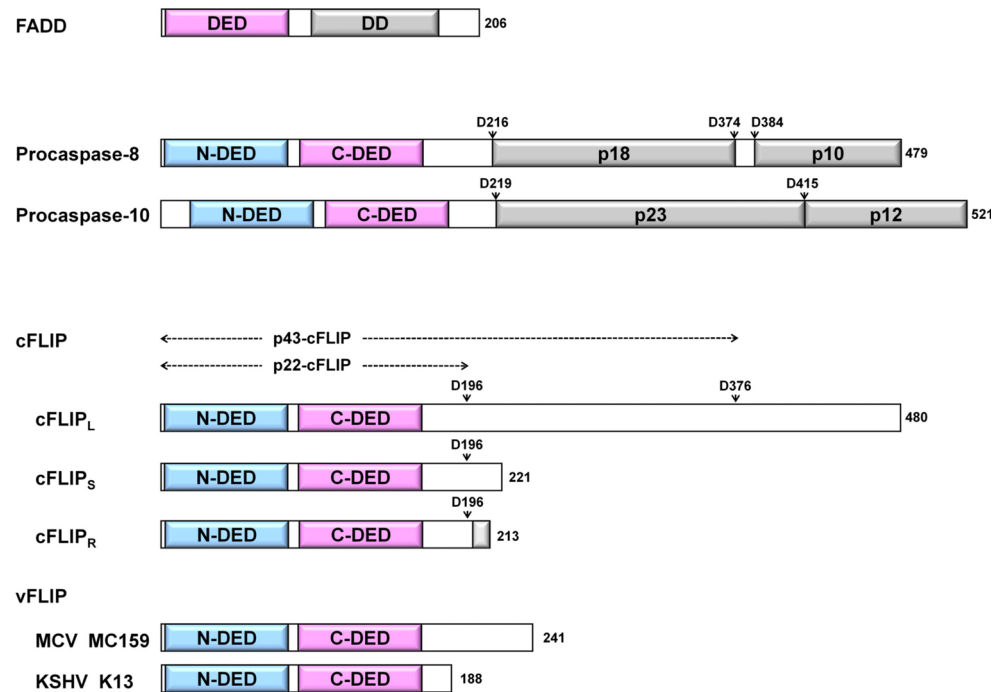
noticed as a common feature of DD superfamily members (Fig. 2A). On the other hand, the structure of tandem DEDs of procaspase-8/-10 or cFLIP has not been analyzed yet. Instead, the crystal structures of two different vFLIPs have been determined: one is MC159 from a poxvirus *Molluscum contagiosum* virus (MCV) [17] and the other is K13 from Kaposi's sarcoma-associated herpesvirus (KSHV) [18]. Crystal structure of MC159-vFLIP presented the first observation on DED–DED interaction even though it is intra-molecular interaction between two DEDs in a single polypeptide. It revealed several important structural features of DED and its homotypic interaction. First, the

Fig. 2 Structures of death effecter domains. **a** FADD DED^{F25Y} (PDB 1A1W). **b** MC159-vFLIP (PDB 2BBR). H1–H4 face and H2–H5 face are noted in *black*, and the other helices are in *gray*. Among the two DEDs in tandem, N-DED is colored in *light blue*, and C-DED in *light pink*. Hydrophobic residues involved in binding of two DEDs are shown as *sticks*

C-terminal DED (C-DED) among the tandem DEDs exhibits the canonical hexa-helical bundle fold just like FADD DED. In contrast, N-terminal DED (N-DED) deviates significantly from the canonical fold: it lacks a helix corresponding to the helix 3 (H3) of the canonical fold and includes an additional helix (H7) after H6. Second, the binding of N-DED and C-DED is established by the interaction between the hydrophobic surfaces of H2–H5 face (N-DED) and H1–H4 face (C-DED). This feature contrasts CARD–CARD binding which is based on the electrostatic interaction between H2 and H3 face (not H2–H5 face) and H1–H4 face as observed in the crystal structure of CARD–CARD complex from Apaf-1 and procaspase-9 [8]. The crystal structure of MC159-vFLIP was used as a reference for the homology modeling studies on the tandem DEDs in procaspase-8 and cFLIP, which predicted very high structural similarity between them [19, 21]. Especially, the involvement of H2–H5 face should be the characteristic element of DED–DED interaction, because the alternative H2–H3 face, instead, is commonly involved in CARD–CARD and in DD–DD interactions [8, 16, 27]. The crystal structure of KSHV-vFLIP (or K13) showed that the above structural features of tandem DEDs are mostly conserved at least for viral FLIPs. Notably, the crystal structure of KSHV-vFLIP was analyzed in a very different functional context from the previously reported MC159-vFLIP structure, because the structure of KSHV-vFLIP was determined in complex with IKKγ component of IKK complex which is a central player in NF-κB signaling [18]. Accordingly, it explained the structural basis of the most important non-apoptotic function of p22-cFLIP

and its viral homolog that is the activation of NF-κB signaling for the survival and proliferation of lymphocytes. The structural and functional features of FLIP for cell survival and proliferation were reviewed elsewhere by Yu et al. [22].

Emerging view on DED interactions in DISC assembly

Structural knowledge on DEDs of DISC components have been limited to individual structures of FADD DED and the viral homolog of cFLIP as reviewed above. Recently, several biochemical studies have provided new insights into the interaction between DED-containing proteins in DISC [19–21]. In these studies, quantitative analyses of mass spectrometry and western blot were used to inspect the stoichiometry of DISC component proteins. Firstly, Dickens et al. [19] found that caspase-8 exists up to 9-fold more than FADD in activated TRAIL DISC. An independent study on Fas DISC by Schleich et al. [20] also showed that caspase-8/cFLIP is more abundant than FADD, but the ratio was about five in western blot and two in mass spectrometry. In another study by Majkut et al. [21], there found approximately two caspase-8/cFLIP molecules recruited for every FADD molecule in TRAIL DISC. Even though the ratio between FADD and caspase-8/cFLIP in DISC is significantly varied among these three independent studies, the common observation is that the adaptor protein FADD exists in a substantially smaller amount than the sum of tandem-DED-containing proteins, caspase-8 and cFLIP. Notably, cFLIP was found in a comparatively small amount in DISC commonly in these studies, reflecting that cFLIP promotes the activation of caspase-8 when its level is low [28]. In addition, caspase-10 exists in DISC in such a negligible level, which may imply that it is not an obligatory component of DISC [19, 31, 32].

The finding of the substoichiometric existence of FADD relative to caspase-8 in DISC led to the proposal that the inter-molecular DED–DED interactions in DISC is not only for between Fadd and procaspase-8/cFLIP but also for the self-association of procaspase-8/cFLIP molecules. This view is well consistent with the previous studies in which DEDs of procaspase-8/-10 and cFLIP was shown to associate with themselves and each other and, moreover, to form a filamentous structure when overexpressed [33, 34]. In order to inspect the surfaces of the inter-molecular DED–DED interactions and finally to build their structural model, homology models of tandem DEDs in procaspase-8 and cFLIP were generated based on MC159-vFLIP crystal structure and then the mutational evaluation was carried out for the possible interface residues [19, 21]. The crystal structure of MC159-vFLIP previously showed that two hydrophobic faces, H2–H5 face and H1–H4 face, are responsible for the intra-molecular DED–DED interaction for tandem DEDs [17]. The homology models for tandem DEDs in cFLIP and procaspase-8 are very
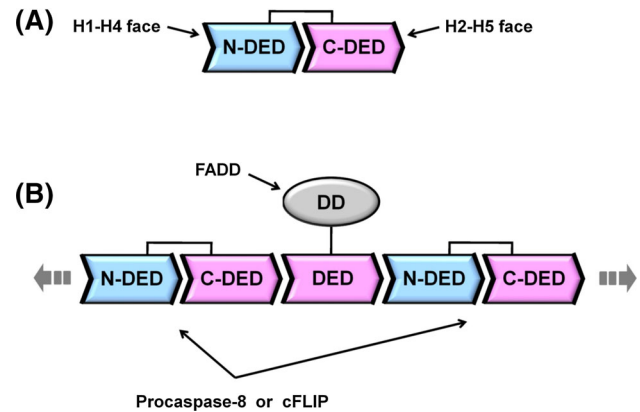
Fig. 3 DED chain model in DISC. a Two different binding faces (H1–H4 face and H2–H5 face) are exposed in tandem DEDs of procaspase-8 and cFLIP. b Proposed topology of DED interaction network in DISC

similar to the MC159-vFLIP structure [19, 21]. The most important point in the homology models of these tandem DEDs is that there are exposed H1–H4 face in N-DED and H2–H5 face in C-DED. The DED chain model argues that the inter-molecular DED–DED interaction in DISC would be established by these two exposed faces which can bind to each other in a very similar way of the intra-molecular DED–DED interaction (Fig. 3). In more detail, the both faces within FADD DED interact with the corresponding partner faces of tandem DEDs in procaspase-8 and cFLIP. Again, the remaining open faces of tandem DEDs in procapase-8 and cFLIP would interacts with other molecules of themselves or less frequently with FADD molecules, thereby establishing namely the DED chain (Fig. 3). Mutations of the hydrophobic interface residues in H2–H5 face (F25 in FADD, F122 and L123 in C-DED of procaspase-8) were shown to abolish the formation of DISC and the activation of caspase-8 [19, 21]. Similarly, F114 of cFLIP significantly diminished its recruitment to DISC [21]. Conclusively, the DED chain model successfully explains the relatively lower abundance of FADD than procaspase-8/cFLIP and also the previous observations of filament formation of over-expressed DEDs [34]. In addition, the results of mutational evaluations also experimentally support this theoretical model [19, 21].

Conclusions

DISC is a platform for initiator caspase activation in extrinsic apoptosis. However, the structural image of DISC has long been elusive because the experimental observation of its structure has been hampered mainly by its complicated interaction networks between component proteins. Regarding to DED interaction profile in DISC, the DED chain model has been recently proposed from the logical synthesis of the stoichiometry data and analogical

inference from the structure of vFLIP and FADD. This emerging view on the DED–DED interaction profile in DISC successfully explains the accumulated biochemical data so far. However, the DED chain model basically relies on the indirect structural information deduced from the isolated structure of vFLIP. Therefore, the currently proposed DED chain model awaits more direct confirmation through experimental structural analysis. But it must be emphasized lastly that independently acquired multiple sets of biochemical data and mutational evaluation results commonly argue the feasibility of the DED chain model as a current best view on DED interaction network in DISC.

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