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Review

Adapter protein – FADD bridges the apoptosis

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Abstract

More than a century ago, the very first adverse human health effects of Fas-associated death domain (FADD) were reported with cell death. It is most well-known role in apoptosis, FADD has also been seen to play a role in other processes including proliferation, cell cycle regulation, and development. It contains two main domains: A C terminal death domain (DD) and an N terminal death effector domain. On stimulation by the Fas ligand, the Fas receptor trimerises. Many receptors, including Fas, contain a cytoplasmic DD and are therefore named death receptors. FADD binds to the DD of this trimeric structure through its DD leads to apoptosis. FADD also plays a role in regulating necroptosis, a process requiring the serine/threonine kinases. Activated caspase 8 cleaves these kinases, inhibiting necroptosis. Application of Taxol is a drug used in anticancer therapies due to its ability to interfere with microtubule assembly, which leads to cell cycle arrest. It has been suggested that inhibition of FADD might work as a potential targeted therapy for drug-resistant ovarian cancer.

Keywords: Apoptosis, drug effect, fas-associated death domain, genetics

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Fas is a member of the tumor necrosis factor receptor 1 (TNFR) family. Its protein structure consists of an extracellular domain with cysteine-rich repeats and a cytoplasmic tail containing a death domain (DD) [1, 2]. The DD is crucial for Fas signal transduction because a single amino acid mutation in this region results in defective Fas function [3]. Mice and humans with Fas defects suffer from severe autoimmune problems characterized by lymphadenopathy, splenomegaly, elevated serum Abs, and the presence of autoantibodies [3, 4]. Death domains are also found in several other apoptosisinducing TNFR family members, including TNFR-1, death receptor - 3, DR4, and DR5 [2, 5, 6].

To elucidate the signal transduction pathway of Fas-mediated apoptosis, several proteins have been isolated that associate with the cytoplasmic tail of Fas. These include the death domain-associated proteins, Fas-associated DD (FADD) 3 [7–9], RIP (receptor interacting protein) [10], Fas-associated protein factor-1 [11], ubiquitin conjugating enzyme [12, 13], and Daxx [14]. Both FADD and RIP also contain a death domain, and over-expression of either of them in cell lines results in apoptosis. Furthermore, FADD phosphorylation of the cAMP-response element binding protein (CREB) and activation of NF-kB and FADD can also initiate the transcription of anti-apoptotic genes [15].

Structure

The FADD gene is located on chromosome 11q13.3 in humans (Fig. 1) and 7 in mice [16]. Mutations in the FADD gene containing locus are frequently observed in human malignancies [17]. For instance, the 11q13 region contains the fibroblast growth factor 3 and 4 genes which are coamplified in melanoma. It also includes the multiple endocrine neoplasia I gene whose mutation leads to tumor development of several endocrine glands including thyroid. Moreover, two genes implicated in leukemia are found in this locus: NUMA1 which is translocated in acute promyelocytic leukemia, and BCL1 which is located very close to the FADD gene and is mutated in B-cell leukemia/

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lymphoma. Although FADD has a central role in multiple receptor-induced cell death as discussed hereafter, no mutation of the FADD gene itself has been reported so far.

Human and mouse FADD genes have the same quite simple organization consisting of two exons (286 bp and 341 bp in humans; 332 bp and 286 bp in mice) separated by a unique intron of approximately 2 kb. Interestingly, no cap site was reported on the human FADD mRNA [16], suggesting a particular regulation of FADD mRNA translation, although this topic has not been further investigated. Human and mouse FADD genes have the same quite simple organization consisting of two exons (286 bp and 341 Human and mouse FADD proteins are very similar (Fig. 2). They consist of 208 and 205 amino acids (AA), respectively, and share 80% similarity and 68% identity [9]. FADD mRNA and protein are almost ubiquitously expressed in fetal and adult tissues, both in humans and mice [8]. Two domains are particularly well conserved between species: The DD at the COO terminus of the protein, and the death effector domain (DED) at the NH2-terminus of the protein [18, 19]. Both domains play a crucial role in transducing the apoptotic signal mediated by death receptors. Furthermore, a single serine (Ser) phosphorylation site essential for determining cell cycle progression is conserved in both species (human Ser 194 [20] and mouse Ser 191 [21]).

Since the first role ascribed to FADD was to transmit apoptotic signals, it was assumed that FADD protein was exclusively localized in the cytoplasm of the cell. However, a nuclear localization sequence (NLS) and a nuclear export sequence (NES) were recently identified in the human FADD protein (Fig. 2), and account for FADD protein expression in the nucleus and the cytoplasm of the cell, respectively [22]. The vast majority of the reports on FADD focused on the cytoplasmic FADD protein because of its pro-apoptotic function. In contrast, the role of the nuclear FADD is much more mysterious. It was recently reported that FADD expression in the nucleus protects cells from apoptosis, but the mechanism implicated in this survival function has not been investigated [22]. On the other hand, it has been shown that FADD could interact within the nucleus of adherent cells with the methyl-CpG binding domain protein 4 (MBD4) [23]. MBD4

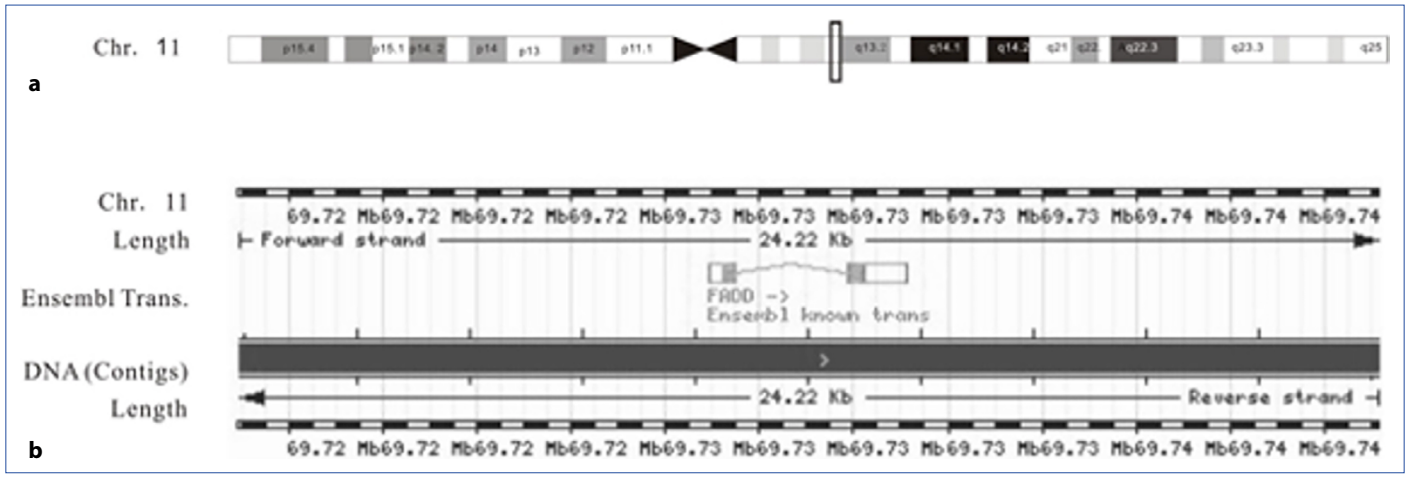

Figure 1. (a) The CCDS8196 (FADD) locus on human chromosome 11Q13. (b) Physical map of the CCDS8196 (FADD) locus on chromosome 16 showing the relative position of FADD gene. This is screen image generated from http://www.ensembl.org/Homo_sapiens/index.html.

FADD: Fas-associated death domain.

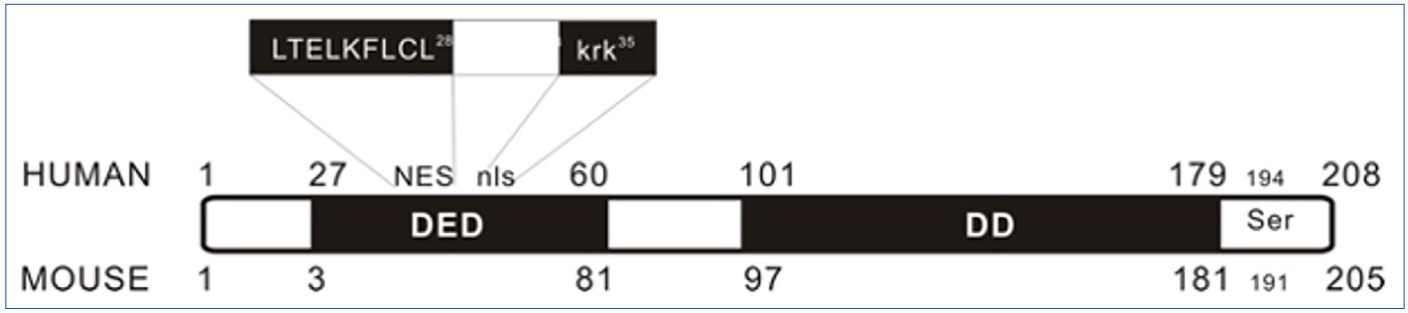

Figure 2. Human and mouse FADD protein. Amino acids (AA) corresponding to the human FADD protein are marked at top, whereas AA corresponding to the mouse FADD protein are marked at bottom. The death domain (DD and death effector domain (DED are essential for interaction with death receptors and transmission of the apoptotic signal. Human nuclear export sequence (NES in Capital letters) and (NLS in Simple letters) determine localization of the protein in the cytoplasm and the nucleus, which are associated with cell death and survival functions of the FADD protein, respectively. Human Ser 194 and mouse Ser 191 phosphorylation site (small in size) have a crucial role in survival/proliferation and cell cycle progression.

FADD: Fas-associated death domain; NLS: Nuclear localization sequence.

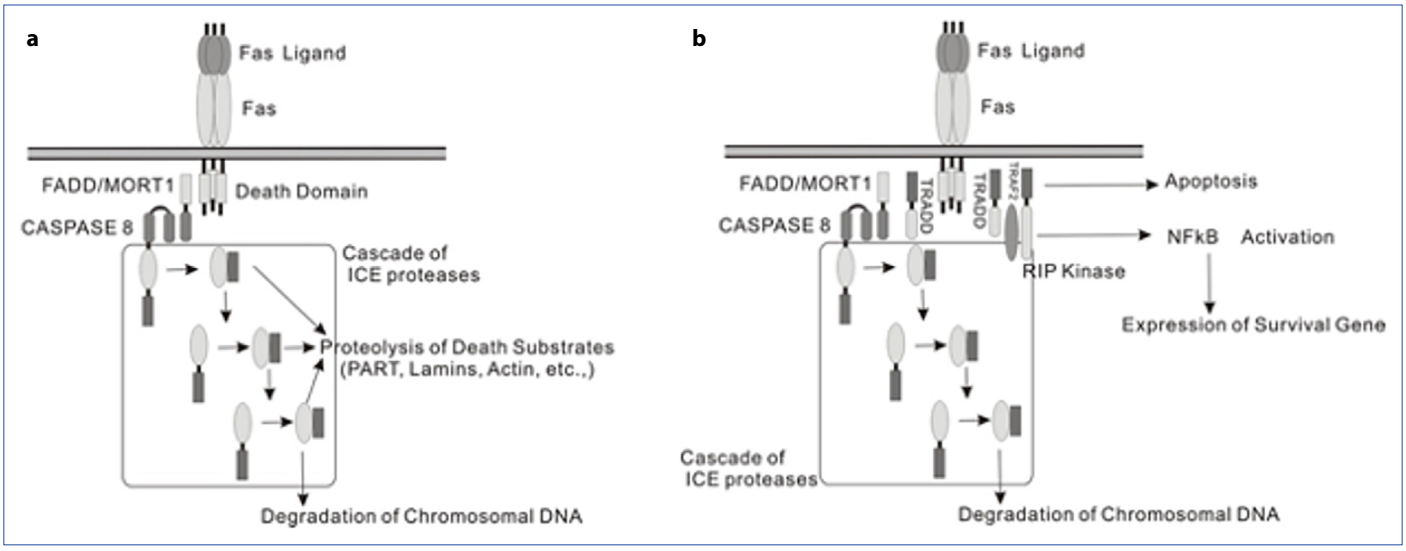

Figure 3. Models for apoptosis signaling by death factors, (a) Fas-induced apoptosis. Binding of FasL to Fas induces trimerization of the Fas receptor, which recruits caspase-8 (FLICE/MACH) through an adaptor, FADD/MORT1. The oligomerization of FLICE may result in self-activation of proteolytic activity and trigger the ICE protease cascade. The activated ICE members can cleave various substrates, such as poly (ADP) ribose polymerase (PARP), lamin, rho-GDI, and actin, and cause morphological changes to the cells and nuclei. (b) TNF-induced apoptosis. TNF binds to TNFR1, and the trimerized receptor recruits TRADD through interactions between death domains. The DD of TRADD then recruits FADD/MORT1 in one pathway to activate caspase-8. In another pathway, RIP binds to TRADD and transduces an apoptotic signal through the death domain. In addition, RIP together with TRAF2 activates NF-kB, which may induce the expression of survival genes. The role of the kinase activity of RIP is currently unknown.

FADD/MORTI: Fas-associated death domain adapter protein; ADP: Adenosine di phosphate; TNFR: Tumor necrosis factor receptor; TRADD: Through another DD.

is a GT mismatch repairing protein. Association between MBD4 and FADD within the nucleus could couple MBD4-mediated genome surveillance with FADD-mediated cell death.

Function

An essential molecule for embryonic development

The essential role of the FADD molecule was highlighted by generating FADD mutant null mice [24, 25]. Indeed, FADD knockout mice were not viable. FADD null embryos died *in utero* at day 12.5 of development, due to underdevelopment, abdominal hemorrhage, and cardiac failure. Moreover, FADD loss of function did not result in a lymphoproliferative disorder as observed in viable Fas mutant mice [3, 26]. These results indicated that in addition to its well-known role in cell death, FADD was also implicated in survival/proliferation of some cell types.

A main death transducer for DD-containing receptors

FADD is the main signal transducing intermediate adaptor molecule of several death receptors including Fas, TNF-R1, DR3 (death receptor 3), TRAIL-R1 (TNF-related apoptosis-inducing ligand, DR4), and TRAIL-R2 (DR5) [8, 24, 25, 27]. All these receptors possess, in their intra-cytoplasmic tail, a DD homologous to the DD of FADD allowing FADD recruitment to the activated receptor. FADD can be recruited either directly to Fas and TRAIL-Rs (Fig. 3a) or indirectly to TNF-R1 (Fig. 3b). In the latter case, FADD is recruited through another DD-containing adaptor molecule (TRADD, TNF receptor-associated protein with DD). Next, FADD recruits DED-containing initiator pro-caspase 8 or 10 through DED/DED interactions [2, 28, 29], thus forming the death-inducing signaling complex (DISC) [30]. Auto processing of initiator pro-caspase leads to activation of effective caspases which cleave intracellular substrates, causing the apoptotic death of the cell [31, 32]. Control of FADD recruitment to the DISC can occur following several mechanisms depending on the cell type and the death receptor [33]. The best characterized death receptor signaling inhibitors are DED-containing viral and cellular FLICE-inhibitory proteins (v-FLIPs and c-FLIPs, respectively) [34, 35]. Inhibition of Fas, TNFR1-, and TRAIL-Rs-induced apoptosis by endogenous FLIPs results from binding of the c-FLIPs to the DED of FADD, thus hindering pro-caspase 8 activation (Fig. 3a). Similarly, v-FLIPs inhibit apoptosis mediated by death receptors either by binding to FADD and blocking pro-caspase 8 processing, or by binding to pro-caspase 8, and inhibiting FADD interaction. Therefore, equilibrium between FADD and the expression of its inhibitors determines the outcome of the death receptor-stimulated cell, that is, apoptosis or survival. All the main death receptors described up to now require FADD adaptor for transmitting their apoptotic signal. Consequently, FADD is a central protein that controls multiple essential cellular processes including cellular homeostasis and elimination of pathological cells, particularly during the course of an immune response.

Death receptor independent FADD-induced apoptosis

Formation of cytoplasmic death effector filaments (DEF) by oligomerization of DED-containing proteins, including

FADD, is responsible for death receptor independent cellular apoptosis [36]. Indeed, FADD over-expression by itself is known to induce cell death through DEF formation that recruits and activates pro-caspase 8. However, the existence of DEF has not been established *in vivo*, and increasing evidences suggest that DEF could be artefactual structures resulting from protein over-expression. As a consequence, the ability of endogenous FADD to aggregate and form DEF in normal situation should be reconsidered.

Gene Regulation

Binding of ligand to a tyrosine kinase receptor, such as PDGF or EGF receptor, induces dimerization of the receptor and activates the intrinsic kinase activity in the cytoplasmic domain. The receptors for hematopoietic growth factors such as colony-stimulating factor and for interferons do not contain kinase domains in their cytoplasmic regions. Instead, the ligand-induced dimerization recruits a kinase(s) to the receptor and activates it, which then results in transduction of the proliferation and/or differentiation signals. In the case of Fas or TNFR1, however, dimerization with a divalent anti-Fas or TNFR1 monoclonal antibody is not sufficient to activate these receptors. Fas and TNFR1 must be oligomerized to be activated; that is, IgM class anti-Fas monoclonal antibody or IgG3 class anti-APO1 antibody that possess a tendency to aggregate function as potent agonists [37, 38]. X-ray diffraction ion analysis of the TNFβ-TNF receptor complex has indicated that a TNFβ trimer makes a complex with three molecules of the extracellular region of the TNF receptor [39], suggesting that TNF induces trilogue) merization of the receptor. The similarity between the structures of FasL and TNF and between Fas and the TNF receptors suggests that FasL also induces trimerization of Fas and that the trimerized cytoplasmic region then transduces the signal.

Utilization of the yeast two-hybrid system with the Fas cytoplasmic region as bait led to the identification of a molecule called FADD or MORT1, which contains a death domain at its C terminus [40, 41]. FADD/MORT1 is recruited to Fas on its activation [38] and binds to Fas through interactions between the death domains. The N-terminal region ([DED] or MORT1 domain) is responsible for downstream signal transduction. A similar death domain-containing protein (TRADD, TNFR1-associated DD protein) binds to TNFR1 [42]. However, unlike FADD/MORT1, TRADD does not carry a DED. Moreover, its DD is responsible for mediating apoptosis. This apparent discrepancy between FADD/MORT1 and TRADD is resolved by the finding that TRADD binds to FADD/MORT1 through interactions between their death domains [43].

These results suggest that Fas and TNFR1 use FADD as a common signal transducer and share the signaling machinery downstream of FADD/MORT1 (Fig. 3). In addition to this pathway, TNFR1 has another pathway leading to apoptosis. RIP, originally identified as a Fas-binding protein, preferentially binds to TRADD [44]. RIP is a serine/threonine kinase containing a DD and binds to TRADD through interactions between their death domains. RIP induces apoptosis when overexpressed. The DD of RIP, but not its kinase domain, is responsible for transduction of the death signal, indicating that RIP does not possess a DED, but rather another downstream effector molecule may be recruited through the DD of RIP (Fig. 3).

To find the signaling molecule downstream of FADD/MORT1, Wallach and his associates again used the yeast two-hybrid system, using the N-terminal DED/MORT1 domain of FADD/MORT1 as bait [45]. At the same time, a collaborative group, led by Dixit and Peter, continued the biochemical characterization of molecules recruited to the activated Fas receptor [31]. Both groups identified the same molecule, which was originally termed FLICE (FADD-like ICE) or MACH (MORT1-associated CED-3 homologue) and is now designated caspase-8. Caspase-8 carries two DED/MORT1 domains at the N-terminal region, through which it binds FADD/MORT1. The C-terminal region of caspase-8 is related to ICE family members, more specifically, cleaves caspase-3 substrates over caspase-1 (ICE) substrates [40].

Figure 3 presents the current model for Fas-and TNFR1-mediated apoptosis. Binding of a trimeric FasL to Fas induces trimerization of Fas, and FADD/MORT1 binds to the trimerized Fas cytoplasmic region through the interaction of the respective death domains. Caspase-8 is then recruited to FADD/MORT1 through binding of the DED domains, which, in turn, may induce self-activation of the protease domain. One apoptotic pathway from TNFR1 uses caspase-8 pathway through the interaction of TRADD with FADD/MORT1. TRADD additionally recruits RIP, which may trigger a second apoptotic pathway. The recently identified DR-3/Wsl-1 receptor is more similar to TNFR1 than to Fas. That is, DR-3 binds TRADD, which then recruits FADD and RIP [8, 46]. The apoptotic signaling pathway downstream of RIP is currently adaptor termed RAIDD (RIP-associated Ich-1/CED-3 homologous protein with a death domain) has recently been identified [47]. RAIDD binds RIP through its DD and recruits caspase-2 (Ich-1) to RIP. Although an involvement of RAIDD in the TNFR1 or DR3/Wsl-1-mediated apoptotic pathway has not yet been demonstrated, it is possible that RAIDD plays a role in transducing an apoptotic signal from one of the death receptors.

FADD and its Phosphorylation

Phosphorylation site of FADD

Human FADD contains two serine clusters, one located at the N and one at the C terminus of the molecular. Phosphorylation of FADD occurs only at a C-terminal serine cluster and is located at serine 194 [20], while mouse FADD is phosphorylated at both serine and threonine residues and the major phosphorylation occurs at the serine residues revealed by 2-D analysis [9]. One of the phosphorylation sites of mouse FADD is serine 191, an amino acid that is equivalent to serine 194 of human FADD. This position is outside the FADD death effector- and death domains, but it is the key to its role in the regulation of growth and proliferation [21]. A point mutant of C-FADD

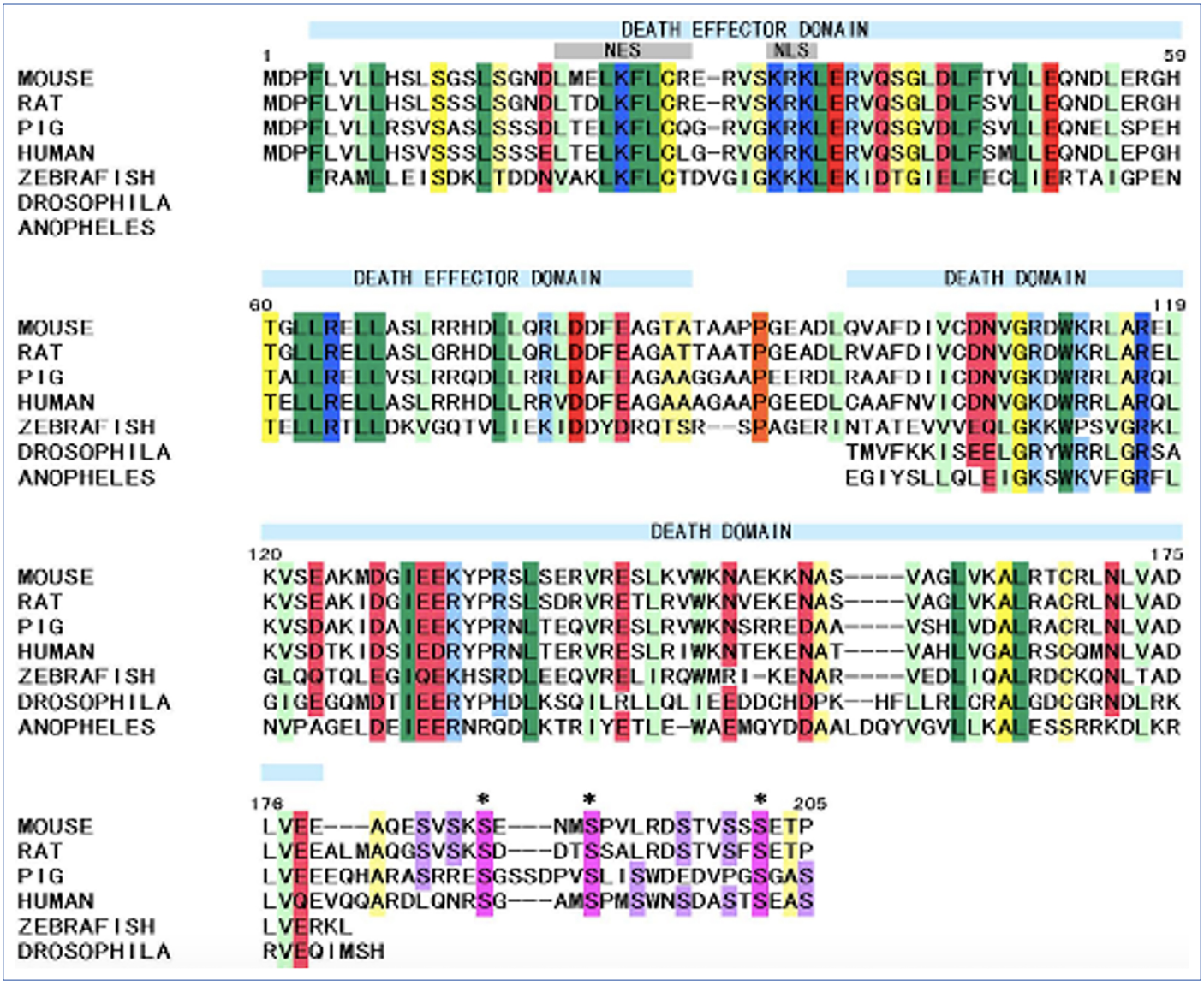

Figure 4. The conserved functional domains and phosphorylation sites of FADDs in seven different species. The alignment was generated using T-coffee and Clustalw and was curated manually. Identical residues that are conserved in the similar property, such as acidic, basic or hydrophobic property, are shown in color shades: hydrophobic residues (V, I, L, F, M, Y, and W) in green; small residues (G, A, S, T, and C) in yellow; proline residues in orange; acidic residues(D, E, Q, and N) in red; basic residues(R, K and H) in blue; the serine (S) in the C-terminal regions in purple. The residue numbers are based on the corresponding amino acid residue position in the mouse FADD protein. DED and DD are indicated in the azury boxes at the top of the sequences. The known phosphorylation site: mouse Ser 191 (equivalent to Ser194 of human FADD) and other two putative ones, Ser 187, Ser 202, are indicated by asterisks to highlight their conservation in mammals. Two regions: NLS, nuclear localization signal and NES, nuclear export signal, are also indicated. Most of FADD protein sequences are obtained from NCBI or Ensembl. The pig and zebrafish FADD sequences are assembled from the EST database *(see Color Plate)*.

FADD: Fas-associated death domain; DED: Death effector domain; NES: Nuclear export sequence; NCBI: National center for biotechnology information; EST: Expressed sequence tags.

changing serine 194 into alanine was no longer phosphorylated in an *in vitro* kinase assay and was also no target for the kinase *in vivo* [20]. Both human and murine FADD are specifically phosphorylated at a single serine residue, suggesting that the site and the function of this serine-specific phosphorylation are conserved between both species. Alignment of FADD proteins from different mammalian species shows a remarkable conservation of serine 191 (mouse) or 194 (human) and its surrounding AA (Fig. 4). Interestingly, while the death effector- and death domains are highly conserved across all species examined, the region containing serine191 or 194 is absent from zebrafish, drosophila, and anopheles FADD proteins (Fig. 4). These data suggest that the C terminal region of the mammalian FADD may represent a domain distinct from its more conserved (and primitive) apoptotic region and this novel domain was acquired by mammals and required during evolution.

The related kinase for phosphorylation of FADD

Regulation of protein activity by phosphorylation is a common mechanism used for a variety of signal transduction pathways. It is well known that phosphorylated and unphosphorylated FADD interact equally well with Fas [21]. Recent reports suggest Fas may also be phosphorylated *in vivo* [48]. Using a series of GST-murine Fas variants, novel proteins, including kinases, were identified that associated specifically with the membrane-proximal, cytoplasmic tail of Fas but not with the death domain. One of these kinases (about 43-kD) phosphorylates FADD [48]. As similar finding has been reported by Tschopp's group, they identified a 130-kD kinase that induces FADD phosphorylation and inhibits Fas-mediated Jun NH2-terminal kinase activation, which is designated Fas-interacting serine/threonine kinase/homeodomain-interacting protein kinase (FIST/HIPK3) as a novel Fas-interacting protein [49]. Moreover, it was also found that protein kinase C (PKC) ζ interacts with FADD *in vivo* and that PKCζ immunoextracts prepared from KG1a cells are able to directly phosphorylate FADD *in vitro* [50]. PKC exerts a protective function against Fas death pathway and contribute to the lack of DISC formation. Hence, it is hypothesized that PKCζ may regulate DISC formation by influencing FADD phosphorylation status which facilitates caspase-8 inhibition and subsequent Fas resistance. In summary, the kinase that is responsible for FADD has not been identified, further studies are necessary to identify the FADD kinase and characterize its function in the signaling pathway.

Functions in proliferation and cell cycle progression

Besides being a main death adaptor molecule, FADD is also required for T cell proliferation. The first evidence of this property of FADD came from observations made in chimeric FADD knockout mice. 5-week-old chimeric FADD-/- mice presented a lack of thymocytes compared to wild type animals, with few or no CD4+ CD8+ double positive thymocytes remaining [25]. Moreover, several groups have demonstrated that FADD deficiency in peripheral T lymphocytes resulted in an inhibition of mitogen-induced T cell proliferation [25, 51]. The mechanism leading to FADD-dependent T cell proliferation did not involve the early events associated with cell proliferation since expression level and functionality of the IL-2 receptor, level of IL-2 secretion, mobilization of intracellular calcium, and activation of NF-kB, p38-MAPK, and p44/ 42-MAPK appeared normal in FADD-/- T lymphocytes [51]. Recent data showed that FADD-/- T lymphocytes entered the cell cycle on mitogenic stimulation, but died during progression through the cell cycle [51]. Therefore, lack of proliferation of FADD-deficient T cells results from defective survival associated with progression through the cell cycle rather than defective activation. Up to now, the molecular pathway implicated in FADD-mediated survival of lymphocytes has not been described.

In addition to impairing survival during cell division, FADD deficiency also leads to a dysregulation of the cell cycle machinery [52]. The pattern of expression of molecules implicated in both G1/S and G2/M transitions was aberrant in FADD-/- lymphocytes, resulting in spontaneous entry and progression through the cell cycle of 10% of freshly isolated FADD-/- T cells (as compared to < 2% of wild type T cells) [52].

The mechanisms responsible for FADD regulation of cell cycle progression are not fully understood. However, the phosphorylation of the 194 human and 191 mice Ser of the protein (Fig. 4) has recently drawn attention. Indeed, human FADD was phosphorylated at Ser 194, by a still unidentified 70 kDa protein kinase, in cells arrested in G2/M, whereas it was unphosphorylated in G1/S [20]. Generation of FADD Ser 191 mutant mice confirmed that FADD phosphorylation is involved in proliferation *in vivo* [21]. Replacement of Ser 191 by an aspartic acid resulting in constitutive phosphorylation of the FADD protein led to abnormal development of FADD mutant mice that shared the same phenotype as FADD deficient mice [8], including few CD4+ CD8+ thymocytes and defective progression through the cell cycle [25, 53].

Tumor cells are constantly cycling cells. Although it does not seem to directly affect the cell cycle progression, FADD phosphorylation at Ser 194 sensitizes these cells to reagents that induce G2/M arrest such as the Taxol anticancer drug [53]. In human prostate cancer cell lines, treatment with Taxol resulted in Ser 194 FADD phosphorylation and G2/M arrest [54]. Moreover, etoposide or cisplatin chemotherapeutic drug-induced apoptosis of these cells was enhanced by pretreatment with Taxol, a process that was inhibited by cellular over-expression of an unphosphorylable FADD mutant [55]. Therefore, tumor cells that express a Ser 194 FADD mutant that cannot be phosphorylated or are unable to phosphorylate FADD at this Ser position are expected to resist apoptosis induced by anticancer drugs that induce G2/M arrest, and to be insensitive to the synergistic effect of chemotherapy. Obviously, a lack of FADD expression will have the same consequences.

FADD and Ovarian Cancer

Ovarian cancer is a type of cancer that starts in the cells of the ovaries, which are the female reproductive glands that produce eggs. Ovarian cancer is often referred to as a "silent killer" because it frequently does not cause noticeable symptoms in its early stages and is often diagnosed at a late stage when it is more difficult to treat.

FADD (Fas-associated protein with death domain) is a protein that is involved in programmed cell death (apoptosis) and is found to be altered in several types of cancer, including ovarian cancer. FADD is a key component of the DISC and is essential for the activation of the caspase cascade, a series of proteases that initiate the apoptotic process. Overexpression or mutation of FADD has been shown to contribute to the development and progression of ovarian cancer, making it a potential target for therapeutic intervention.

The clinical importance of FADD in ovarian cancer lies in its role as a potential therapeutic target. As mentioned earlier,

altered expression or function of FADD has been implicated in the development and progression of ovarian cancer. Therefore, targeting FADD or its downstream signaling pathways could have a potential therapeutic benefit in treating ovarian cancer.

Several clinical studies have investigated the potential of targeting FADD in ovarian cancer treatment. These studies have explored the use of small molecule inhibitors, RNA interference, and antisense oligonucleotides to target FADD or its downstream signaling pathways. In preclinical studies, these approaches have shown promising results in reducing the growth and survival of ovarian cancer cells.

However, despite the promising results of preclinical studies, the clinical application of FADD targeting in ovarian cancer treatment is still in its early stages. Further clinical studies, including larger scale clinical trials, are needed to fully evaluate the efficacy and safety of FADD-targeting approaches in the treatment of ovarian cancer.

In conclusion, FADD plays a critical role in the development and progression of ovarian cancer, making it a promising target for therapeutic intervention. While further studies are needed to fully assess its potential, FADD holds a place as a potential target in the treatment models of ovarian cancer.

There are several methods used for the analysis of FADD in relation to ovarian cancer:

- Immunohistochemistry (IHC) – This is a method used to detect the presence of specific proteins, including FADD, in tissue samples. In IHC, tissue sections are treated with antibodies specific to the target protein (in this case FADD) and then visualized using a detection system. IHC can be used to determine the expression levels of FADD in ovarian cancer tissues and to assess its potential as a prognostic marker or therapeutic target.
- Western blotting – This is a method used to measure the amount of specific proteins in a sample. In Western blotting, protein extracts from tissue samples are separated by size using gel electrophoresis, and then transferred onto a membrane. The membrane is then treated with antibodies specific to the target protein (in this case FADD) and visualized using a detection system. Western blotting can be used to determine the expression levels of FADD in ovarian cancer tissues and to assess its potential as a therapeutic target.
- Reverse transcription polymerase chain reaction (RT-PCR) – This is a method used to measure the amount of RNA in a sample. RT-PCR can be used to determine the expression levels of FADD in ovarian cancer tissues and to assess its potential as a therapeutic target.
- Flow cytometry – This is a method used to analyze the expression levels of proteins in individual cells. In flow cytometry, cells are labeled with antibodies specific to the target protein (in this case FADD) and then analyzed using a flow cytometer. Flow cytometry can be used to determine the expression levels of FADD in ovarian cancer cells and to assess its potential as a therapeutic target.

These methods can provide important information on the role of FADD in ovarian cancer and help in the development of targeted therapeutic strategies.

Conclusion

FADD is a protein that has been implicated in the development and progression of ovarian cancer. Its role as a key component of the DISC makes it a promising target for therapeutic intervention in the treatment of ovarian cancer. While further studies are needed to fully assess its potential, FADD holds promise as a potential target in the treatment of ovarian cancer. The analysis of FADD expression levels in ovarian cancer tissues and cells can provide important information on its role in the disease and help in the development of targeted therapeutic strategies. The future studies should focus on exploring the mechanisms underlying the regulation of FADD, evaluating the efficacy and safety of FADD-targeting therapeutic approaches, and developing new and more specific FADD-targeting strategies.

Future studies

FADD holds great promise as a therapeutic target in the treatment of ovarian cancer, as its altered expression or function has been implicated in the development and progression of the disease. The future studies should focus on further exploring the role of FADD in ovarian cancer and the mechanisms underlying its regulation.

In particular, the future studies should aim to:

1. Investigate the mechanisms by which FADD contributes to the development and progression of ovarian cancer, including its role in cell survival, proliferation, and apoptosis.
2. Evaluate the efficacy and safety of FADD-targeting therapeutic approaches, such as small molecule inhibitors, RNA interference, and antisense oligonucleotides, in preclinical models and clinical trials.
3. Develop new and more specific FADD-targeting strategies to enhance the therapeutic potential of FADD-directed therapy.
4. Assess the potential of FADD as a prognostic marker and a predictor of response to therapy in ovarian cancer patients.

Overall, the future studies on FADD and ovarian cancer have the potential to improve our understanding of the disease and to provide new therapeutic approaches for the treatment of ovarian cancer.

Conflict of Interest: None declared.

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Peer-review: Externally peer-reviewed.

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