Apoptosis (2019) 24:385–394
https://doi.org/10.1007/s10495-019-01540-0

REVIEW

Modulation of CD95-mediated signaling by post-translational modifications: towards understanding CD95 signaling networks

Kamil Seyrek[1] · **Inna N. Lavrik**[1]

Published online: 8 May 2019

Abstract

CD95 is a member of the death receptor family and is well-known to promote apoptosis. However, accumulating evidence indicates that in some context CD95 has not only the potential to induce apoptosis but also can trigger non-apoptotic signal leading to cell survival, proliferation, cancer growth and metastasis. Despite extensive investigations focused on alterations in the expression level of CD95 and associated signal molecules, very few studies, however, have investigated the effects of post-translational modifications such as glycosylation, phosphorylation, palmitoylation, nitrosylation and glutathionylation on CD95 function. Post-translational modifications of CD95 in mammalian systems are likely to play a more prominent role than anticipated in CD95 induced cell death. In this review we will focus on the alterations in CD95-mediated signaling caused by post-translational modifications of CD95.

Keywords CD95 · Glycosylation · Phosphorylation · Palmitoylation · Nitrosylation · Glutathionylation

Abbreviations

DR	Death receptor
TNF-R	Tumor necrosis factor receptor
CD95	Cluster of differentiation 95
FADD	Fas-associated protein with death domain
c-FLIP	Cellular FLICE inhibitory protein
DISC	Death-inducing signaling complex
DD	Death domain
DED	Death effector domain
MOMP	Mitochondrial outer membrane permeabilisation
Bid	BH3 interacting-domain death agonist
tBid	Truncated Bid
ERK	Extracellular signal-regulated kinase
NF-κB	Nuclear factor κB
CD95L	CD95 ligand
CRD	Cysteine-rich domain
NK	Natural killer
EGFR	Epidermal growth factor receptor
EGF	Epidermal growth factor
pEGFR	Phosphorylated EGFR
pSTAT3	Phosphorylated STAT3
SHP-1	Src homology domain 2 (SH2)-containing tyrosine phosphatase-1
NO	Nitric oxide

✉ Inna N. Lavrik
inna.lavrik@med.ovgu.de

[1] Translational Inflammation Research, Institute of Experimental Internal Medicine, Medical Faculty, Otto von Guericke University, Magdeburg, Germany

Introduction

Apoptosis is a form of programmed cell death that eliminates damaged and excessive cells to maintain tissue homeostasis. There are two key ways of apoptosis induction: the extrinsic and the intrinsic pathway [1, 2]. The intrinsic pathway is initiated via UV- or gamma-irradiation, growth factor withdrawal and genotoxic stress. This leads to mitochondrial outer membrane permeabilisation (MOMP), which is followed by the release of several pro-apoptotic factors from the mitochondria resulting in the initiation of the cell death cascade. The extrinsic cell death pathway is triggered by stimulation of the death receptors (DRs). Six DR family members have been characterized so far: TNF-R1, CD95 (FAS/APO-1), DR3, TRAIL-R1/DR4, TRAIL-R2/DR5, and DR6 [3]. Among the DR family, CD95 is one of the best-characterized members [4]. CD95 is present on the surface of most cell types. In particular, pancreas, kidney liver, heart, thymus, lymphoid tissue and mature T cells express high levels of CD95 on their cell surface. On the contrary, expression of CD95 ligand (CD95L) is highly restricted to

natural killer (NK) cells and activated T cells [5]. CD95L can be either present in the membrane-bound form, or can be cleaved off from the membrane by metalloproteinases and released in a soluble form [6].

CD95 comprises the N-terminal extracellular (aa 1–170), transmembrane (171–198) and C-terminal intracellular parts. The extracellular part of CD95 is characterized by the presence of three cysteine-rich domains (CRDs), while the intracellular part of CD95 contains so called Death Domain (DD) [3]. The presence of a DD in the intracellular region is a key feature of all members of DR family that plays a major role in the assembly of macromolecular complexes and subsequent signal transduction [3]. Activation of CD95 is triggered by binding of CD95L or agonistic antibodies to the extracellular region of CD95 in the vicinity of CRD2 and CRD3. This leads to conformational changes of CD95 in the transmembrane and intracellular domains that allow the recruitment of Fas-associated death domain protein (FADD). Subsequently, procaspase-8a/b, -10a/d, or c-FLIP$_{L/S/R}$ are recruited to form the death-inducing signaling complex (DISC) [7]. In the assembly of the DISC, FADD plays a central role by serving as a major link between DR triggering and procaspase-8 activation. DD of FADD binds to DD of CD95, while the death effector domain (DED) of FADD interacts with DEDs of procaspase-8a/b, -10a/d, or c-FLIP$_{L/S/R}$ [8, 9]. Activation of procaspase-8 at the CD95 DISC occurs via dimerization at the DED filaments. DED filaments at the DISC are formed via interactions of DEDs of procaspase-8 upon its recruitment to FADD [10, 11]. The structural mechanisms of DED filament assembly and function are only starting to be uncovered. Procaspase-8 activation leads to apoptosis by activating downstream proteins [12] (Fig. 1).

Upon activation in the DED filaments, procaspase-8a/b undergoes autocatalytic processing which involves two steps. At the first auto-proteolytic cleavage step p43/p41 and p12 subunits are generated followed by the formation of the subunits p18, p10 and the prodomains p26/p24 [13]. In addition, there are less abundant cleavage products formed at the DED filaments that include the C-terminal procaspase-8a/b cleavage product p30 and the N-terminal elongated prodomain (CAP3) [14–16]. The generated active caspase-8 heterotetramer $p10_2$–$p18_2$ is disseminated into the cytosol leading to the activation of downstream effector caspases-3 and -7 [13, 17].

Two distinct CD95 signaling pathways have been described. Type I cells show a high level of DISC formation and caspase-8 activation upon CD95 stimulation. In Type II cells, however, because of the low level of DISC generation amplification of the apoptotic signal is required. To this end, the BH3 interacting-domain death agonist (Bid) is cleaved by procaspase-8 to generate truncated Bid (tBid). This leads to MOMP and a subsequent release of proapoptotic factors from the mitochondria. The release of cytochrome C from mitochondria results in apoptosome formation followed by activation of procaspase-9, which in turn activates downstream effector caspases leading to amplification of the apoptotic signal [18, 19].

CD95 has not only the potential to induce apoptosis but can also trigger non-apoptotic signals [20]. In certain cell types CD95 may induce migration, proliferation or cytokine production. In particular, CD95 has been shown to be implicated in neuronal regeneration, liver regeneration and antigen-stimulated T cells proliferation [5, 21–24]. As a receptor with pleiotropic functions CD95 has also been described to activate nuclear factor κB (NF-κB) transcription in tumour cell lines [14, 19, 20, 25–27]. A cancer-promoting function of CD95 is recognized, though the proliferative potential of CD95 has been neglected for quite a while [28–30].

The induction of life and death signals via CD95 requires intricate regulation of the macromolecular complexes assembled at CD95. In particular, CD95 requires a conformational change in its DD for the efficient recruitment of FADD, procaspase-8, -10 and c-FLIP, followed by DED filament assembly [31]. Despite intensive studies the key molecular mechanisms of DISC formation and DED chain assembly that define induction of apoptotic to non-apoptotic signals are not fully understood. However, apart from several reported mechanisms that are largely based on protein–protein interactions, post-translational modifications of CD95 may play an important role in defining the molecular details of induction of apoptotic or non-apoptotic pathways. Indeed, post-translational modifications of CD95 might modulate the strength of interaction of CD95 with CD95L and adaptor proteins, define the degree of its oligomerization and promote or inhibit the possibility for conformational changes of CD95 leading to DED filament activation. Recently, several post-translational modifications of CD95 have been identified. Among a large number of regulatory mechanisms of apoptosis, post-translational modifications such as glycosylation, palmitoylation, phosphorylation, nitrosylation, and glutathionylation may play a key role in the determination of DR signaling activation and in particular of CD95 [32, 33].

Hence, in this review we will focus on the modulation of signaling attributed to post-translational modifications in CD95.

Post-translational glycosylation of CD95

Protein glycosylation is an enzyme-directed and site specific complex process affecting the folding, stability, subcellular localization and biological activity of proteins [34]. Glycosylation profiles of proteins also influence the immune response, cell signaling pathways and cell–cell recognition of various cell types [34, 35]. Aberrant glycosylation

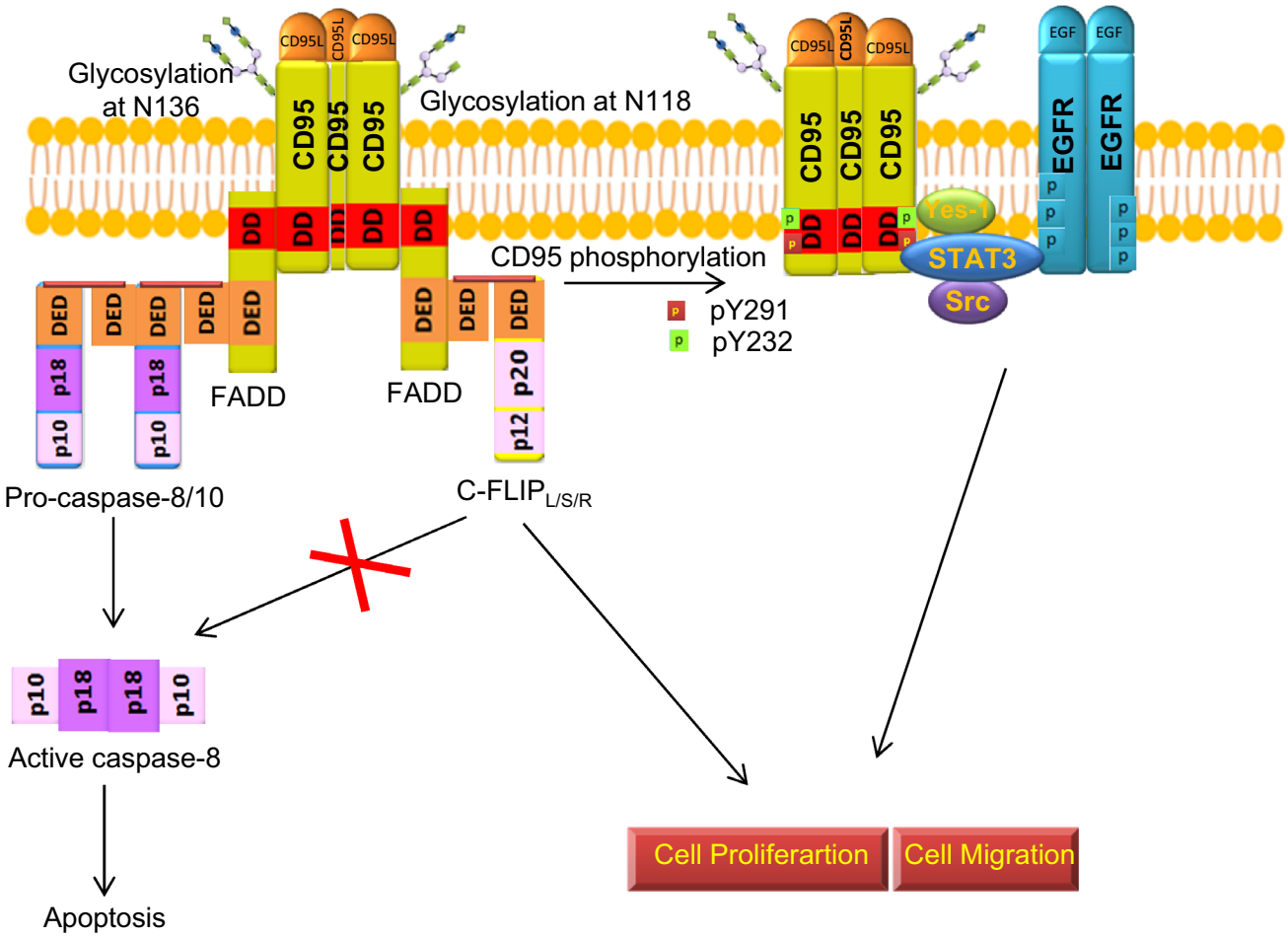

Fig. 1 CD95-induced apoptotic pathway and modulation of CD95 signaling by glycosylation and phosphorylation. Upon activation of CD95 with CD95L or agonistic antibodies, the death-inducing signaling complex (DISC) is formed. Activation of caspase-8 occurs at DED filaments formed at the DISC, which provide a platform for procaspase-8 dimerization. The subsequent autocatalytical cleavage of procaspase-8 generates active caspase-8 heterotetramer with two p18 and two p12 subunits. Caspase-8 activates downstream caspase cascade including caspase-3 (casp3). CD95 is N-glycosylated at two sites, N118 and N136, in the extracellular domain. Furthermore, CD95 is phosphorylated at Y232 and Y291, which are localized within the DD of the receptor. Phosphorylation at Y291 activates the pro-survival signals. Furthermore, the phosphorylated form of CD95 at Y291 was reported to interact with epidermal growth factor receptor (EGFR) leading to the generation of a complex consisting of CD95, EGFR, Yes-1, Src, and STAT3

of proteins is often connected with the development and progression of several diseases such as immune deficiencies, neurodegeneration and cancer [34, 35]. Sugar moieties can be attached to proteins either through a bond between *N*-acetylglucosamine (GlcNAc) and asparagine referred to as N-linked or through a link between *N*-acetylgalactosamine (GalNAc) and the hydroxyl groups of serine or threonine referred to as *O*-linked [36]. There is ample evidence linking apoptosis and glycosylation. Glycans have been shown to control the secretion or expression of cell surface receptors. They may also control the interactions between receptors and their ligands [37, 38]. In particular, glycosylation of CD95 might have several key regulatory functions. Translocation of CD95 to the plasma membrane is a prerequisite for ligand association and transduction of apoptotic signaling [37]. Furthermore, glycosylation may crucially control the interactions between CD95 and its ligand as well as define the degree of CD95 oligomerization, the latter playing an important role in the regulation of CD95 response. Finally, there is increasing evidence that the molecular architecture of CD95/CD95L complexes plays a key role in the induction of downstream signaling. The conformation of CD95 and CD95L in these complexes might be largely fine-tuned by glycosylation. Accordingly, the delineation of CD95 glycosylation is essential for getting insights into CD95-mediated signaling.

CD95 is a glycoprotein modified with both *O*- and *N*-linked glycans in its extracellular domain [37, 39]. The extracellular domain of CD95 contains two *N*-glycosylation sites at positions N118 and N136 [40], whereas in

the intracellular domain of CD95 two other glycosylation sites were predicted: a potential site of *N*-glycosylation at the position N223 and an O-glycosylation site [40]. Native CD95 has a molecular mass of about 35 kDa, but after post-translational modifications, mature CD95 is mostly expressed as glycosylated proteins of about 45–52 kDa [28, 41]. Due to the difference in glycosylation pattern CD95 can appear in several glycoforms depending on the particular cell type [40]. Because of the proximity of the *N*-glycosylation sites, N118 and N136, to its ligand binding sites, it is conceivable to expect that *N*-glycans may influence the biological activity of CD95 [40]. Therefore, the role of *N*-glycosylation in the binding of CD95L, and subsequently its contribution to the stabilization of the DISC structure, DISC–DISC interactions and oligomerization of procaspase-8 at DISC are very important topics.

There have been several studies addressing these questions. In particular, in T cells it has been shown that defects in CD95 glycosylation block CD95 oligomerization resulting in the abrogation of the signal [42]. Furthermore, interestingly, a difference in CD95 glycosylation pattern between Type I and Type II cells has been reported. Type I cells, such as T lymphoma cells Hut78 as well as B lymphoblastoid cells SKW6.4 and BJAB have two major glycoforms of CD95 with different molecular mass, while Type II cells like Jurkat and CEM cells, mostly have one major CD95 form [40]. Higher amounts of DISC in Type I cells than Type II cells may indicate that the stronger DISC formation may be attributed to the presence of specific carbohydrate structures at CD95 in Type I cells.

In many cell types CD95 was reported to be sialylated on the N-linked oligosaccharide residues [39, 43]. CD95-sialylation has been described both to inhibit and promote CD95 apoptotic activity. In particular, α2-6 sialylation of CD95 has been reported to inhibit DISC formation and suppress CD95 internalization but does not interfere with binding of the agonistic anti-CD95 antibodies [44]. Desialylation of CD95 using *Vibrio cholera* neuraminidase in various human B and T cell lines increases the sensitivity of these cells towards CD95-induced apoptosis [39, 43]. Contrary to these findings Shatnyeva et al. reported that CD95 desialylation in B lymphoblastoid SKW6.4 and T leukemia Hut78 cells by *Vibrio cholera* neuraminidase reduces DISC formation without affecting CD95 surface expression and its binding affinity to anti-APO-1 antibodies [40]. Taken together, these reports strongly suggest that sialylation/desialylation of CD95 contributes to the regulation or modulation of CD95 signaling. However, association between the degree of CD95 sialylation and the strength of CD95 signaling still has to be uncovered. Furthermore, discrepancies between different studies should be addressed in the future studies.

Taken together, glycosylation of CD95 seem to play a key role in CD95 signaling control. However, further studies are required to address the role of glycosylation in life and death decisions at CD95. Finally, an important issue will be the analysis of CD95 glycosylation in different cell types and the correlation of glycostructure to the strength of CD95L-induced pro- and anti-apoptotic signals. These studies are highly relevant for deciphering the mechanisms of sensitivity and resistance of cancer cells towards apoptosis.

Phosphorylation of CD95

Protein phosphorylation is carried out through protein kinases by adding a phosphate group to the target protein at serine, threonine, or tyrosine residues. Phosphorylation of membrane receptors is one of the key mechanisms in signal transduction. Similar to other membrane proteins human CD95 is predicted to have several serine, threonine, or tyrosine phosphorylation sites in its intracellular domain [40]. The particular attention in CD95 phosphorylation network has been given to CD95 phosphorylation at tyrosines Y232 and Y291, which are localized within the DD of the receptor [45] (Fig. 1). It has been reported that tyrosine phosphorylation in the DD of CD95 blocks the pro-apoptotic signal by inhibiting FADD recruitment, DISC formation and apoptosis. Especially, phosphorylation at Y291 is reported to turn CD95 into a pro-survival state [46]. In particular, Src- and Yes- induced CD95 DD phosphorylation leads to inhibition of apoptosis and promotes cell proliferation and migration in breast, colon and ovarian cancers [45, 46]. Furthermore, CD95 phosphorylation is described to promote neuron regeneration and promote tumor growth in glioblastoma [47–50]. In addition, CD95 phosphorylation at Y291 has been shown to mediate autophosphorylation of epidermal growth factor receptor (EGFR) leading to the interaction between CD95 and EGFR and formation of a complex that consists of CD95, EGFR, Yes-1, Src, and STAT3 [45] (Fig. 1). In this complex, the phosphorylated form of CD95 serves as a hub for the recruitment of EGFR, STAT3 and Yes-1. Interestingly, once the cells are activated by epidermal growth factor (EGF) phosphorylated CD95 is reported to translocate to the nucleus, where it promotes the nuclear localization of phosphorylated EGFR (pEGFR), phosphorylated STAT3 (pSTAT3), and the expression of cyclin D1 resulting in proliferation and migration of the cells [45]. Furthermore, the negative charge caused by phosphorylation at Y291 has been shown to promote clathrin-mediated endocytosis of CD95 upon CD95L binding [46].

Phosphorylation of DR is a reversible process. Dephosphorylation of CD95 by Src homology domain 2 (SH2)-containing tyrosine phosphatase-1 (SHP-1) has been recently shown to positively regulate CD95-mediated apoptosis and negatively regulate survival and proliferation of cells [51–54]. In particular, in colorectal cancer cells SHP-1 has

been reported to act as a pro-apoptotic modulator based on the removal of phosphate groups in the DD of CD95 [46, 51]. However, SHP-1 activity might depend on cellular context, and species. For example, SHP-1 deficiency in mice did not alter CD95-mediated apoptosis in the lymphoid organs but in human hepatocytes and thymocytes in the absence of SHP-1 apoptosis mediated by CD95 has been reported to be abrogated [51, 52, 55].

Contrary to the abovementioned investigations, other studies revealed that a phosphorylation of CD95 DD is critical for targeting of the CD95/EGFR complex to the plasma membrane, DISC formation and apoptosis induction [56, 57]. In human Huh7 hepatoma cells it has been shown that the presence of phosphate groups at Y232 and Y291 is not a prerequisite for CD95 association with EGFR but that these phosphate groups in DD are essential for targeting of the EGFR/CD95 complex to the plasma membrane, subsequent DISC formation and apoptosis induction [56, 57]. Eberle et al. have reported that the EGFR/CD95 complex is assembled in the cytosol and subsequently targeted to the cell membrane [56]. At the cell membrane EGFR/CD95 complex is reported to act as a platform for DISC assembly. Replacement of tyrosine with phenylalanine at positions 232 and 291 has revealed that phosphorylation of CD95 is not essential for the EGFR/CD95 association, whereas CD95 mutants failed to be targeted to the plasma membrane, to recruit FADD and caspase-8 and to induce apoptosis [56]. The possible explanation of the discrepancy concerning the role of phosphorylation in DDs of CD95 could be the different cell types used in the different studies which asks for a cell type-specific analysis of DR phosphorylation in future investigations. Another point, which has to be clarified in future studies is CD95 presence in different cellular compartments, as the larger part of the CD95 cellular pool has been reported to be targeted to the plasma membrane. Hence, the mechanisms of cellular localization of CD95 have to be further unraveled. Although there are some contradictory results, phosphorylation seems to be the important point in the CD95 signaling network defining whether a pro-survival or an apoptotic signal is initiated at CD95.

Taken together, phosphorylation of DD of CD95 seems to present an important regulatory node for signal regulation. Indeed, the changes in the conformation of CD95 DD that are induced by phosphorylation might inhibit the recruitment of adaptor proteins like FADD and therefore define the signaling outcome. However, it is important to note that CD95L-mediated FADD recruitment occurs very fast, e.g. within seconds after CD95 stimulation [7, 14]. Accordingly, if one assumes that both alternative processes, phosphorylation of DD by tyrosine kinases and FADD recruitment to DD, occur in parallel then the balance between apoptotic *versus* anti-apoptotic signaling should directly depend on the binding affinity of FADD *versus* tyrosine kinases to the DD of CD95, and the abundance of these proteins. This further highlights the necessity of getting a quantitative understanding of the CD95 phosphorylation network in different cell types, which would undoubtedly unveil further details of life/death decisions at CD95.

Palmitoylation of CD95

Palmitoylation is the covalent attachment of a 16-carbon saturated fatty acid, palmitate, to sulfhydryl groups of cysteine residues located at intracellular regions of transmembrane proteins [5, 58]. Due to the hydrophobic character of palmitic acid, the features of the protein are profoundly influenced by binding of this hydrocarbon derivate. In particular, depending on the hydrophobic or hydrophilic character of the surrounding membrane lipids or proteins, interactions of a palmitoylated protein with surrounding molecules can be promoted or constrained. In this way palmitoylation influences protein properties such as interactions with specific membrane domains, trafficking between cellular compartments or stability [59, 60].

Similar to other transmembrane proteins CD95 has been shown to be reversibly palmitoylated. Palmitoylation occurs in the intracellular region at cysteine 199 in human CD95 and cysteine 194 in murine CD95. Palmitoylation of CD95 at cysteine 199 has been shown to be essential for the oligomerization of CD95 and the subsequent assembly of CD95 microaggregates at the cell membrane [32]. CD95 microaggregates are reported to promote caspase-8 activation, receptor internalization and apoptosis induction (Fig. 2). In this way, this modification seems to be crucial for the proper anchoring of CD95 at the plasma membrane and induction of downstream signals.

Palmitoylation at Cys199 has also been reported to mediate CD95 translocation within the cell membrane to lipid rafts [61]. Lipid rafts are characterized by the presence of high amounts of sphingolipids and cholesterol, which supports the translocation of the proteins with hydrophobic groups to the lipid rafts [62]. There has been a number of reports that CD95 translocation to lipid rafts plays an important role in induction of CD95-mediated apoptosis [32, 61, 63–66]. Interestingly, a constitutive residence of CD95 in lipid rafts in Type I cells but not in Type II cells seems to result in more effective DISC formation in Type I cells [62, 66].

It is also tempting to speculate that restricted lateral diffusion of CD95 in lipid rafts, compared to the fluid cytoplasmic membrane, would favor CD95 oligomerization and apoptotic signaling. From another side, there is also strong evidence that the formation of microaggregates and the DISC assembly efficiently occur both within lipid

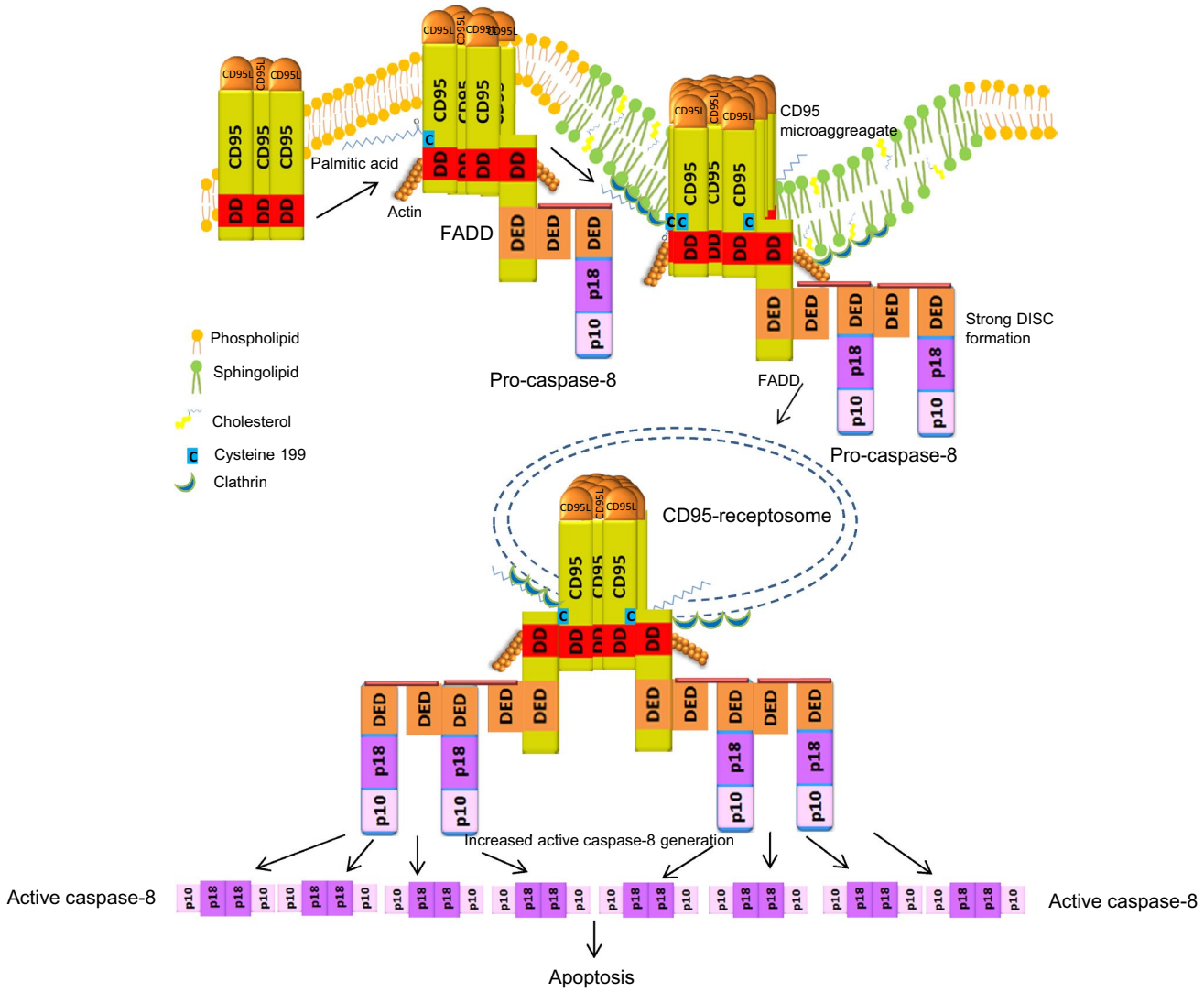

Fig. 2 Promoting of CD95-mediated apoptosis via palmitoylation of death receptor. CD95 palmitoylation occurs in the intracellular region at cysteine 199 (Cys199) of human CD95. Subsequently, CD95 forms microaggregates, which promote caspase-8 activation, resulting in apoptosis induction

rafts as well as independently [32]. Hence, the exact role of lipid rafts in CD95 signaling still has to be elucidated. However, it has to be underlined that CD95 with mutation at Cys199 failed to be palmitoylated and generate sufficient amount of DISC for induction of CD95-mediated cell death. This shows the important role of palmitoylation in the proper localization of CD95 at the cell membrane for signal initiation, despite contradictory reports on the role of lipid rafts in CD95-induced apoptosis [32, 61, 62]. Overall, palmitoylation seems to play a crucial role in the initiation and transduction of the CD95-mediated apoptotic signaling. However, more detailed mechanisms of this process remain to be uncovered.

S-nitrosylation of CD95

As a short-lived free radical nitric oxide (NO) acts as a messenger and an effector molecule. NO is generated by several cell types in humans and NO production is essential for numerous biological processes within the cells. NO has been shown to play a critical role in apoptosis [67]. The covalent binding of NO moiety to a cysteine residue in a protein is referred to as S-nitrosylation [67]. NO has been shown to trigger S-nitrosylation of Cys199 and Cys304 in the DD of CD95, which is a reversible process [67]. S-nitrosylation of CD95 was implemented in the regulation of CD95-mediated signaling [67]. In

particular, CD95-nitrosylation correlates with increased CD95 membrane localization and sensitization of cancer cells to CD95-mediated apoptosis [68]. Moreover, cells with CD95 mutated at nitrosylation sites were no more able to be sensitized by NO towards CD95-mediated apoptosis [68].

Interestingly, CD95 nitrosylation occurs both at Cys199 and Cys304; however, only nitrosylation at Cys304, but not at Cys199 seems to determine the CD95 aggregation and DISC formation [68]. Moreover, nitrosylation of CD95 at Cys304 has been reported to be tightly associated with the recruitment of CD95 into lipid rafts [68]. Taken together, nitrosylation of CD95 regulates its activity; however, detailed molecular mechanisms of how CD95-nitrosylation promotes aggregation of this receptor are largely unknown.

Although in the abovementioned studies NO has been reported to act as a pro-apoptotic molecule, according to other reports NO seems to be also an inhibitor of CD95-mediated apoptosis. In particular, in trophoblast cells inhibition of NO synthesis caused a significant increase in receptor clustering, following stimulation with anti-CD95 agonistic antibodies [69]. Furthermore, it is tempting to speculate that inhibition of NO synthesis may also prevent nitrosylation of other core components of the DISC leading to caspase-8 activation. Collectively, CD95 nitrosylation might be an important modulator of CD95-mediated signaling. The observed discrepancies in the pro- *versus* anti-apoptotic role of *S*-nitrosylation may reflect differences in experimental design and a cell type used in different studies. Future work should explicitly clarify the role of nitrosylated CD95 on apoptotic signaling.

S-glutathionylation of CD95

Oxidation of cysteine residues by free oxygen radicals disturbs the functions of proteins. Cellular proteins can function properly only with their cysteines in the reduced state. Under normal conditions, intracellular milieu provides sufficient protection to keep cysteine residues in the reduced thiol state, however in cells undergoing oxidative stress cysteine residues are oxidized. Glutathione is the main molecule maintaining the reduced state of thiol groups in the cell. Glutathione protects proteins against irreversible oxidation by forming reversible disulfide bridges [70]. S-glutathionylation is the generation of a disulfide bridge between the cysteine moiety of a protein and the cysteine of glutathione [71, 72]. CD95 has been shown to be glutathionylated at the carboxyterminal end of DD at Cys294 [71, 72]. This event seems to play a proapoptotic role. Due to the negative charge of glutamic acid, S-glutathionylation of CD95 might result in an enhanced recruitment of FADD and stabilisation of the DISC complex, which has to be checked in future studies.

S-glutathionylation of CD95 is negatively regulated by thiol transferases such as glutaredoxin 1, which reduces the disulfide bridges. Interestingly, glutaredoxin 1 is proteolytically degraded by caspase-8 and/or -3. Thus, in the course of apoptosis, the caspase-mediated cleavage of glutaredoxins blocks further release of glutathione from CD95, which maintains CD95 in its glutationylated form and further amplifies the apoptotic cell death (Fig. 3). In mice with acute *P. aeruginosa* infection glutaredoxin 1 has been shown to be highly upregulated which leads to apoptosis inhibition in epithelial cells through a number of mechanisms that also might involve CD95 glutathionylation [71]. Taken together, S-glutathionylation represents a redox-based modification of cysteines, representing a regulatory switch that affects the strength of CD95-mediated signaling.

Conclusions

Post-translational modifications play a key role in the regulation of signaling networks. A number of signaling pathways are largely driven via phosphorylation and ubiquitinylation such as MAPK and NF-κB signaling, while the major driving post-translational modification in apoptosis is proteolysis which is mediated by caspases. Hence, the question arises what is the contribution of the other non-proteolysis type post-translational modifications to apoptosis onset? Are those essential for apoptosis or anti-apoptosis regulation or just play a role in fine-tuning cell death by slightly modulating the cell death signal? The induction of life and death signals via CD95 requires an intricate regulation of the macromolecular complexes assembled at CD95. Hence, it might be assumed that at the stage of initiation of the apoptotic signal the proper conformation of the core DISC components is a prerequisite for the initiation of cell death. In this regard, an optimal conformation of the DD of CD95 for the binding of FADD DD should be playing a major role in cell fate. Therefore, it is logical to suggest that the post-translational modifications of CD95 play the role of "guardians" of this process, each of them targeting a specific site in CD95 and thereby providing a fine regulation of initiation of a pro or an anti-apoptotic signal. In particular, glycosylation targets CD95 to the plasma membrane and supports the proper architecture of the CD95/CD95L complex. Phosphorylation of the CD95 DD prevents the binding of FADD and blocks apoptosis. Palmitoylation, nitrosylation and glutathionylation of CD95 seem to be responsible for the proper anchoring of CD95 and accordingly its DD at the cell membrane as well as contribute to the oligomerization of CD95, which are prerequisites for apoptosis induction. Hence, the current advances in knowledge indicate that post-translational modifications such as palmitoylation, nitrosylation and glutathionylation

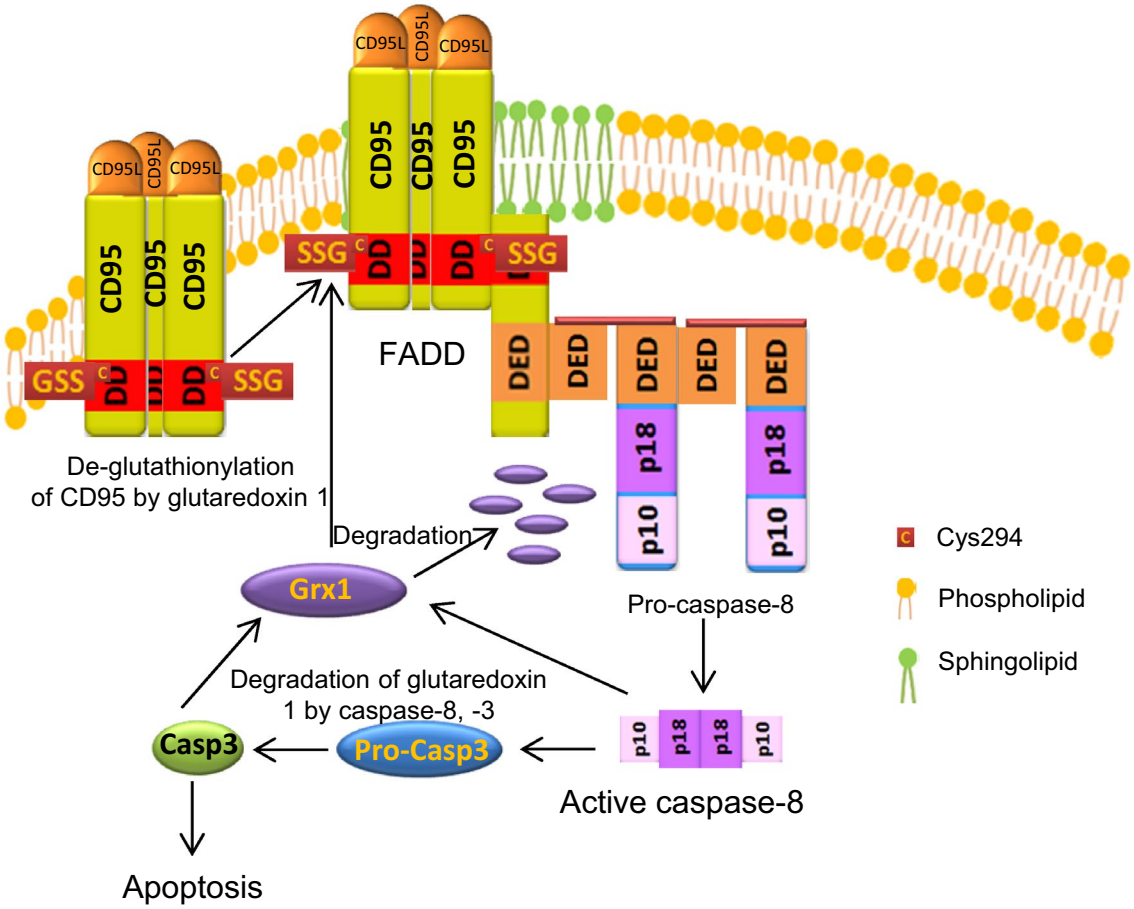

Fig. 3 Glutathion promotes apoptotic cell death. CD95 can be glutathionylated at the carboxyterminal end of DD at Cys294. S-glutathionylation of CD95 is reported to promote the assembly of DISC. Glutaredoxin 1(Grx1) is a thiol transferase that removes glutathione from CD95. Glutaredoxin 1 can be cleaved by caspases, which results in amplification of CD95-mediated apoptotic signal

promote DISC assembly, whereas phosphorylation dampens DISC activation.

Of note there is a very strong controversy in the reports on the role of several post-translational modifications. These controversial results obtained in different studies remain a subject for future research. In this regard, it is especially important to find out whether there are cell type specific differences in the role of a particular post-translational modification. Another important subject of future research involves the analysis of the common sites for post-translational modifications like Cys199, which has been reported to be targeted by both palmitoylation and nitrosylation. Another interesting region of CD95 is 291–294, which involves both sites for phosphorylation (291) and glutathionylation (294). Modification at one site might change the conformation of this region and therefore block the second modification. This opens up a possibility of cross-talk between post-translational modifications which defines the cell fate. Hence, the analysis of these common sites and the cross-talk between different post-translational modifications will undoubtedly bring new insights on the mechanism of the decisions between life and death. Finally, CD95 has been reported to induce in parallel both apoptosis and NF-κB pathways [27, 73]. The molecular mechanisms of this induction still have to be unraveled. It is tempting to speculate that yet to be discovered post-translational modifications of CD95 can be responsible for mediating these two signaling routes.

Taken together, post-translational modifications of CD95 are just starting to be uncovered and deciphering their role will undoubtedly present a new powerful code for understanding the life and death decisions in a cell as well as will open new ways for developing therapies associated with defects in CD95 regulation.

Acknowledgements We acknowledge Volkswagen Foundation (VW 90315), Wilhelm Sander-Stiftung (2017.008.01), Center of dynamic systems (CDS), funded by the EU-programme ERDF (European Regional Development Fund) and DFG (LA 2386) for supporting our work.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

1. Elmore S (2007) Apoptosis: a review of programmed cell death. Toxicol Pathol 35:495–516
2. Igney FH, Krammer PH (2002) Death and anti-death: tumour resistance to apoptosis. Nat Rev Cancer 2:277–288
3. Lavrik I, Golks A, Krammer PH (2005) Death receptor signaling. J Cell Sci 118:265–267
4. Gloire G, Charlier E, Piette J (2008) Regulation of CD95/APO-1/Fas-induced apoptosis by protein phosphatases. Biochem Pharmacol 76:1451–1458
5. Sessler T, Healy S, Samali A, Szegezdi E (2013) Structural determinants of DISC function: new insights into death receptor-mediated apoptosis signalling. Pharmacol Ther 140:186–199
6. Schulte M, Reiss K, Lettau M et al (2007) ADAM10 regulates FasL cell surface expression and modulates FasL-induced cytotoxicity and activation-induced cell death. Cell Death Differ 14:1040–1049
7. Kischkel FC, Hellbardt S, Behrmann I et al (1995) Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor. EMBO J 14:5579–5588
8. Algeciras-Schimnich A, Griffith TS, Lynch DH, Paya CV (1999) Cell cycle-dependent regulation of FLIP levels and susceptibility to Fas-mediated apoptosis. J Immunol 162:5205–5211
9. Thome M, Schneider P, Hofmann K et al (1997) Viral FLICE-inhibitory proteins (FLIPs) prevent apoptosis induced by death receptors. Nature 386:517–521
10. Dickens LS, Boyd RS, Jukes-Jones R et al (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
11. Schleich K, Warnken U, Fricker N et al (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
12. Krammer PH, Arnold R, Lavrik IN (2007) Life and death in peripheral T cells. Nat Rev Immunol 7:532–542
13. Scaffidi C, Medema JP, Krammer PH, Peter ME (1997) FLICE is predominantly expressed as two functionally active isoforms, caspase-8/a and caspase-8/b. J Biol Chem 272:26953–26958
14. Golks A, Brenner D, Krammer PH, Lavrik IN (2006) The c-FLIP-NH2 terminus (p22-FLIP) induces NF-kappaB activation. J Exp Med 203:1295–1305
15. Hoffmann JC, Pappa A, Krammer PH, Lavrik IN (2009) A new C-terminal cleavage product of procaspase-8, p30, defines an alternative pathway of procaspase-8 activation. Mol Cell Biol 29:4431–4440
16. Golks A, Brenner D, Schmitz I et al (2006) The role of CAP3 in CD95 signaling: new insights into the mechanism of procaspase-8 activation. Cell Death Differ 13(3):489–498
17. Charlier E, Conde C, Zhang J et al (2010) SHIP-1 inhibits CD95/APO-1/Fas-induced apoptosis in primary T lymphocytes and T leukemic cells by promoting CD95 glycosylation independently of its phosphatase activity. Leukemia 24:821–832
18. Korsmeyer SJ, Wei MC, Saito M, Weiler S, Oh KJ, Schlesinger PH (2000) Pro-apoptotic cascade activates BID, which oligomerizes BAK or BAX into pores that result in the release of cytochrome c. Cell Death Differ 7:1166–1173
19. Lavrik IN, Golks A, Riess D, Bentele M, Eils R, Krammer PH (2007) Analysis of CD95 threshold signaling: triggering of CD95 (FAS/APO-1) at low concentrations primarily results in survival signaling. J Biol Chem 282:13664–13671
20. Hayden MS, Ghosh S (2012) NF-kappaB, the first quarter-century: remarkable progress and outstanding questions. Genes Dev 26:203–234
21. Cullen SP, Henry CM, Kearney CJ et al (2013) Fas/CD95-induced chemokines can serve as "find-me" signals for apoptotic cells. Mol Cell 49:1034–1048
22. Kennedy NJ, Kataoka T, Tschopp J, Budd RC (1999) Caspase activation is required for T cell proliferation. J Exp Med 190:1891–1896
23. Lavrik IN (2014) Systems biology of death receptor networks: live and let die. Cell Death Dis 5:e1259
24. Newton K, Harris AW, Bath ML, Smith KG, Strasser A (1998) A dominant interfering mutant of FADD/MORT1 enhances deletion of autoreactive thymocytes and inhibits proliferation of mature T lymphocytes. EMBO J 17:706–718
25. Barnhart BC, Legembre P, Pietras E, Bubici C, Franzoso G, Peter ME (2004) CD95 ligand induces motility and invasiveness of apoptosis-resistant tumor cells. EMBO J 23:3175–3185
26. Bentele M, Lavrik I, Ulrich M et al (2004) Mathematical modeling reveals threshold mechanism in CD95-induced apoptosis. J Cell Biol 166:839–851
27. Buchbinder JH, Pischel D, Sundmacher K, Flassig RJ, Lavrik IN (2018) Quantitative single cell analysis uncovers the life/death decision in CD95 network. PLoS Comput Biol 14:e1006368
28. Neumann L, Pforr C, Beaudouin J et al (2010) Dynamics within the CD95 death-inducing signaling complex decide life and death of cells. Mol Syst Biol 6:352
29. Siegmund D, Lang I, Wajant H (2017) Cell death-independent activities of the death receptors CD95, TRAILR1, and TRAILR2. FEBS J 284:1131–1159
30. Yuan K, Jing G, Chen J et al (2011) Calmodulin mediates Fas-induced FADD-independent survival signaling in pancreatic cancer cells via activation of Src-extracellular signal-regulated kinase (ERK). J Biol Chem 286:24776–24784
31. Lavrik IN, Krammer PH (2012) Regulation of CD95/Fas signaling at the DISC. Cell Death Differ 19:36–41
32. Feig C, Tchikov V, Schutze S, Peter ME (2007) Palmitoylation of CD95 facilitates formation of SDS-stable receptor aggregates that initiate apoptosis signaling. EMBO J 26:221–231
33. Jin Z, Li Y, Pitti R et al (2009) Cullin3-based polyubiquitination and p62-dependent aggregation of caspase-8 mediate extrinsic apoptosis signaling. Cell 137:721–735
34. Very N, Lefebvre T, El Yazidi-Belkoura I (2018) Drug resistance related to aberrant glycosylation in colorectal cancer. Oncotarget 9:1380–1402
35. Munkley J, Elliott DJ (2016) Hallmarks of glycosylation in cancer. Oncotarget 7:35478–35489
36. Ferreira JA, Magalhaes A, Gomes J et al (2017) Protein glycosylation in gastric and colorectal cancers: toward cancer detection and targeted therapeutics. Cancer Lett 387:32–45
37. Li Y, Yang X, Nguyen AH, Brockhausen I (2007) Requirement of N-glycosylation for the secretion of recombinant extracellular domain of human Fas in HeLa cells. Int J Biochem Cell Biol 39:1625–1636
38. Brockhausen I (2006) The role of galactosyltransferases in cell surface functions and in the immune system. Drug News Perspect 19:401–409
39. Peter ME, Hellbardt S, Schwartz-Albiez R et al (1995) Cell surface sialylation plays a role in modulating sensitivity towards APO-1-mediated apoptotic cell death. Cell Death Differ 2:163–171

40. Shatnyeva OM, Kubarenko AV, Weber CE et al (2011) Modulation of the CD95-induced apoptosis: the role of CD95 N-glycosylation. PLoS ONE 6:e19927
41. Garcia-Fuster MJ, Ferrer-Alcon M, Miralles A, Garcia-Sevilla JA (2004) Deglycosylation of Fas receptor and chronic morphine treatment up-regulate high molecular mass Fas aggregates in the rat brain. Eur J Pharmacol 496:63–69
42. Lichtenstein RG, Rabinovich GA (2013) Glycobiology of cell death: when glycans and lectins govern cell fate. Cell Death Differ 20:976–986
43. Keppler OT, Peter ME, Hinderlich S et al (1999) Differential sialylation of cell surface glycoconjugates in a human B lymphoma cell line regulates susceptibility for CD95 (APO-1/Fas)-mediated apoptosis and for infection by a lymphotropic virus. Glycobiology 9:557–569
44. Swindall AF, Bellis SL (2011) Sialylation of the Fas death receptor by ST6Gal-I provides protection against Fas-mediated apoptosis in colon carcinoma cells. J Biol Chem 286:22982–22990
45. Ta NL, Chakrabandhu K, Huault S, Hueber AO (2018) The tyrosine phosphorylated pro-survival form of Fas intensifies the EGF-induced signal in colorectal cancer cells through the nuclear EGFR/STAT3-mediated pathway. Sci Rep 8:12424
46. Chakrabandhu K, Hueber AO (2016) Fas versatile signaling and beyond: pivotal role of tyrosine phosphorylation in context-dependent signaling and diseases. Front Immunol 7:429
47. Corsini NS, Sancho-Martinez I, Laudenklos S et al (2009) The death receptor CD95 activates adult neural stem cells for working memory formation and brain repair. Cell Stem Cell 5:178–190
48. Kleber S, Sancho-Martinez I, Wiestler B et al (2008) Yes and PI3 K bind CD95 to signal invasion of glioblastoma. Cancer Cell 13:235–248
49. Letellier E, Kumar S, Sancho-Martinez I et al (2010) CD95-ligand on peripheral myeloid cells activates Syk kinase to trigger their recruitment to the inflammatory site. Immunity 32:240–252
50. Sancho-Martinez I, Martin-Villalba A (2009) Tyrosine phosphorylation and CD95: a FAScinating switch. Cell Cycle 8:838–842
51. Chakrabandhu K, Huault S, Durivault J et al (2016) An evolution-guided analysis reveals a multi-signaling regulation of fas by tyrosine phosphorylation and its implication in human cancers. PLoS Biol 14:e1002401
52. Su X, Zhou T, Wang Z, Yang P, Jope RS, Mountz JD (1995) Defective expression of hematopoietic cell protein tyrosine phosphatase (HCP) in lymphoid cells blocks Fas-mediated apoptosis. Immunity 2:353–362
53. Daigle I, Yousefi S, Colonna M, Green DR, Simon HU (2002) Death receptors bind SHP-1 and block cytokine-induced anti-apoptotic signaling in neutrophils. Nat Med 8:61–67
54. Duchesne C, Charland S, Asselin C, Nahmias C, Rivard N (2003) Negative regulation of beta-catenin signaling by tyrosine phosphatase SHP-1 in intestinal epithelial cells. J Biol Chem 278:14274–14283
55. Singer BB, Klaile E, Scheffrahn I et al (2005) CEACAM1 (CD66a) mediates delay of spontaneous and Fas ligand-induced apoptosis in granulocytes. Eur J Immunol 35:1949–1959
56. Eberle A, Reinehr R, Becker S, Haussinger D (2005) Fluorescence resonance energy transfer analysis of proapoptotic CD95-EGF receptor interactions in Huh7 cells. Hepatology 41:315–326
57. Eberle A, Reinehr R, Becker S, Keitel V, Haussinger D (2007) CD95 tyrosine phosphorylation is required for CD95 oligomerization. Apoptosis 12:719–729
58. Dietrich LE, Ungermann C (2004) On the mechanism of protein palmitoylation. EMBO Rep 5:1053–1057
59. Blaskovic S, Blanc M, van der Goot FG (2013) What does S-palmitoylation do to membrane proteins? FEBS J 280:2766–2774
60. Rossin A, Durivault J, Chakhtoura-Feghali T, Lounnas N, Gagnoux-Palacios L, Hueber AO (2015) Fas palmitoylation by the palmitoyl acyltransferase DHHC7 regulates Fas stability. Cell Death Differ 22:643–653
61. Chakrabandhu K, Herincs Z, Huault S et al (2007) Palmitoylation is required for efficient Fas cell death signaling. EMBO J 26:209–220
62. Ramaswamy M, Cleland SY, Cruz AC, Siegel RM (2009) Many checkpoints on the road to cell death: regulation of Fas-FasL interactions and Fas signaling in peripheral immune responses. Results Prob Cell Differ 49:17–47
63. Yurchenko M, Shlapatska LM, Sidorenko SP (2012) The multilevel regulation of CD95 signaling outcome. Exp Oncol 34:153–159
64. Mollinedo F, Gajate C (2006) Fas/CD95 death receptor and lipid rafts: new targets for apoptosis-directed cancer therapy. Drug Resist Updat 9:51–73
65. Pennarun B, Meijer A, de Vries EG, Kleibeuker JH, Kruyt F, de Jong S (2010) Playing the DISC: turning on TRAIL death receptor-mediated apoptosis in cancer. Biochim Biophys Acta 1805:123–140
66. Muppidi JR, Siegel RM (2004) Ligand-independent redistribution of Fas (CD95) into lipid rafts mediates clonotypic T cell death. Nat Immunol 5:182–189
67. Iyer AK, Azad N, Wang L, Rojanasakul Y (2008) Role of S-nitrosylation in apoptosis resistance and carcinogenesis. Nitric Oxide 19:146–151
68. Leon-Bollotte L, Subramaniam S, Cauvard O et al (2011) S-nitrosylation of the death receptor fas promotes fas ligand-mediated apoptosis in cancer cells. Gastroenterology 140:2009–2018 **(2018. e2001-2004)**
69. Dash PR, McCormick J, Thomson MJ, Johnstone AP, Cartwright JE, Whitley GS (2007) Fas ligand-induced apoptosis is regulated by nitric oxide through the inhibition of fas receptor clustering and the nitrosylation of protein kinase Cepsilon. Exp Cell Res 313:3421–3431
70. Janssen-Heininger YM, Aesif SW, van der Velden J et al (2010) Regulation of apoptosis through cysteine oxidation: implications for fibrotic lung disease. Ann N Y Acad Sci 1203:23–28
71. Anathy V, Aesif SW, Guala AS et al (2009) Redox amplification of apoptosis by caspase-dependent cleavage of glutaredoxin 1 and S-glutathionylation of Fas. J Cell Biol 184:241–252
72. Fernandes AP, Holmgren A (2004) Glutaredoxins: glutathione-dependent redox enzymes with functions far beyond a simple thioredoxin backup system. Antioxid Redox Signal 6:63–74
73. O' Reilly LA, Tai L, Lee L et al (2009) Membrane-bound Fas ligand only is essential for Fas-induced apoptosis. Nature 461:659–663