Targeting c-FLIP in cancer

Sarah Shirley[*], Olivier Micheau [*‡¶]

[*] INSERM, U866, Dijon, F-21079 France ; Faculty of Medicine and Pharmacy, Univ. Bourgogne, Dijon, F-21079 France.

[‡] Centre Georges-François Leclerc, Dijon, F-2100 France.

[¶] Correspondence should be addressed to: O Micheau, INSERM, U866, Dijon, F-21079 France; Faculty of Medicine and Pharmacy, Univ Bourgogne, Dijon, F-21079 France. Phone: + 33 3 80 39 34 68. Fax: + 33 3 80 39 34 34; e-mail: omicheau@u-bourgogne.fr.

Keywords: c-FLIP, Death Receptors, Cancer.

Abbreviations: CDDP, cisplatin; VP16, etoposide; 5FU, 5-Fluorouracil; DISC, Death-Inducing Signaling Complex; DcR2, TRAIL-R4 or Decoy Receptor 2; DR5, TRAIL-R2; DR4, TRAIL-R1.

Abstract

Cellular-FLICE inhibitory protein (c-FLIP) is a key anti-apoptotic regulator that inhibits cell death mediated by the death receptors Fas, DR4, DR5, and TNFR1. Three splice variants of c-FLIP function at the DISC level by blocking the processing and activation of procaspase-8 and -10. Overexpression of c-FLIP has been identified in many different tumor types, and its downregulation in vitro has been shown to restore apoptosis mediated by CD95L and TRAIL. c-FLIP therefore represents a promising target for cancer therapy. This review focuses on the molecular mechanisms that control c-FLIP expression and current research into inhibitors of the protein. Increasing evidence supports the investigation of c-FLIP as a therapeutic target to restore an apoptotic response in cancer cells.

1 Introduction 3
2 c-FLIP 3
2.1 c-FLIP isoforms 3
2.2 c-FLIP functions 4
2.3 Overexpression of cFLIP in tumors 5
3 Transcriptional regulation of c-FLIP expression 6
4 Post-translational regulation of c-FLIP expression 8
5 Targeting c-FLIP for cancer therapy 9
5.1 Transcriptional regulators 10
5.2 Genetic approaches : siRNAs 11
5.3 Targeting c-FLIP for degradation 11
6 Conclusions 12

1 Introduction

Apoptosis is crucial for tissue homeostasis and normal development. Two major signaling pathways engage the apoptotic program: the mitochondrial-dependent intrinsic pathway that is activated upon intracellular signals, and the extrinsic pathway that is triggered upon binding of ligands such as TRAIL, Fas, and TNFα with their cognate death receptors at the cell surface. Ligand binding induces oligomerization of the receptors, allowing the formation of macromolecular complexes formed by homotypic interactions by means of the Death Domain (DD) and Death Effector Domains (DEDs) of adaptor proteins such as FADD or TRADD, intitiator caspases, the procaspase-8 and -10. Binding of Fas ligand and TRAIL to death receptors recruits initiator caspases to the membrane, within the death-inducing signaling complex (DISC) (Figure 1A). Upon assembly of the DISC, the dimerization and transproteolytic cleavage of procaspase-8 results in the release of the catalytically active p10-p18 subunit heterotetramer [1]. Recruitment and activation of initiator caspases upon TNF-R1 engagement takes place in complex II, a cytosolic complex generated sequentially from TNF-R1 DISC, which at the membrane contains TRADD and RIP but lacks FADD and caspase-8 [2]. This event triggers a proteolytic cascade, leading to the activation of executioner caspases-3 and -7, which drive apoptotic cell death (Figure 1A).

2 c-FLIP

2.1 c-FLIP isoforms

c-FLIP, also known as Casper, iFLICE, FLAME-1, CASH, CLARP, MRIT or usurpin, is a crucial negative regulator of the apoptotic pathway [3]. Thirteen splice variants of the gene have been identified, but only three of these have been shown to be translated to protein. These are c-

$FLIP_L$, c-$FLIP_S$, and c-$FLIP_R$ (Figure 1B). c-$FLIP_L$ is a 55 kDa protein that is structurally similar to procaspase-8, with two N-terminal DED domains and a C-terminal caspase-like domain [4]. The C-terminal domain of c-$FLIP_L$ lacks the catalytic cysteine residue, which confers the proteolytic activity of caspases. c-$FLIP_L$ is thus devoid of enzymatic activity. c-$FLIP_S$ (26 kDa) and c-$FLIP_R$ (24 kDa) also contain two N-terminal DEDs, but with a shorter carboxy terminus than c-$FLIP_L$. The C-terminal tails of the short forms of c-FLIP play an important role in the ubiquitylation and degradation of the proteins, as well as contributing to apoptotic function [5; 6]. All three isoforms of c-FLIP can be recruited to the DISC through an interaction of their tandem DED domains with the adaptor protein FADD.

2.2 c-FLIP functions

Recruitment of c-$FLIP_S$ to the DISC or to complex II inhibits procaspase-8 dimerization and activation, thus blocking the activation of the apoptotic cascade. c-$FLIP_L$, on the other hand, forms a heterodimeric complex with caspase-8, but unlike c-$FLIP_S$, heterodimerization of c-$FLIP_L$ with procaspase-8 induces caspase-8 activation in the absence of cleavage [7]. Limited activation of caspase-8 in these complexes results in the generation of p43-c-FLIP and the p41/43 caspase-8 subunits. However, as no further processing occurs due to the lack of proteolytic activity of c-$FLIP_L$, cleaved products remain bound at the DISC, preventing further transduction of the apoptotic signal (Figure 1A). Nevertheless, active caspase-8 has access to a limited subset of substrates, including RIP [7]. Differential cleavage RIP at the membrane, could therefore account for the differential regulation of downstream signaling pathways such as c-Fos or NF-kB by c-$FLIP_L$ and c-$FLIP_S$ upon Fas ligand stimulation [8]. A puzzling detail with respect to the inhibitory potential of this protein, is the finding that c-$FLIP_L$ expression levels in some tumor cells are sometimes much lower than the levels of caspase-8 itself [9], yet c-FLIP clearly protects

against ligand mediated cell death. This inhibitor appears in fact to be preferentially recruited within the DISC [10] or complex II [2], with caspase-8. The molecular mechanisms behind this preferential recruitment are unknown, but since the heterodimer c-FLIP$_L$-Caspase-8 is more stable than the homodimer [7; 10], it cannot be excluded that the heteromers may stabilize DISC formation more efficiently than the caspase-8 homodimers, but this hypothesis awaits further investigation.

2.3 Overexpression of cFLIP in tumors

c-FLIP has been found at elevated levels in a number of different cancers. Studies of cell lines have demonstrated increased levels of c-FLIP in colorectal carcinoma [11], gastric adenocarcinoma [12], pancreatic carcinoma [13], melanoma [14], ovarian carcinoma [15], and prostate carcinoma [16]. Studies in primary tissues from patients have also demonstrated that there are increased levels of c-FLIP in malignant cells in B-cell chronic lymphocytic leukemia [17; 18], bladder urothelial carcinoma [19], lung adenocarcinoma [20], gallbladder carcinoma [21] and hepatocellular carcinoma [22]. Analysis of primary cells from patients also confirmed the upregulation of c-FLIP in gastric carcinoma [23; 24], Hodgkins lymphoma [25], non small cell lung carcinoma [26], and melanoma [27]. It is of interest to note that in primary Ewing sarcoma, including metastases, c-FLIP was shown to be abundantly expressed in 18 of 18 patients [28].

In the majority of cases it is the c-FLIP$_L$ isoform that is overexpressed in malignancy, however there are some studies showing upregulation of c-FLIP$_S$. Gastric carcinoma SNU-216 cells demonstrated high levels of c-FLIP$_S$ [12], as did pancreatic cancer cell lines [29]. Tissue samples from lung adenocarcinoma patients also showed an overexpression of c-FLIP$_S$, but not c-

$FLIP_L$ [20].

Overexpression of c-FLIP is associated with an increased resistance to apoptosis mediated by Fas and TRAIL, and studies have demonstrated that in some tissue types, high levels of c-FLIP expression correlates with a more aggressive tumor [30]. Studies of patients with colorectal carcinoma [31], cervical carcinoma [32], Burkitt's lymphoma [33], non-Hodgkin's lymphoma [34], and bladder urothelial carcinomas [19] have demonstrated that elevated levels of c-FLIP in tumor tissue is correlated with a poor prognosis. To our knowledge only one study suggested that c-FLIP levels did not have any correlation with survival in ovarian cancers [35]. Altogether these findings demonstrate that c-FLIP isoforms are often found to be overexpressed in tumors. Their expression levels should thus not only be considered with regard to death receptor targeted therapies, but also to conventional chemotherapy since c-FLIP was shown to inhibit anticancer drug-induced cell death in preclinical models [36; 37].

3 Transcriptional regulation of c-FLIP expression

c-FLIP is a transcriptional target of several transcription factors including NF-κB [38; 39], p53 [40], p63 [41], the forkhead transcription factor FOXO3a [42], EGR1 [43], AR [44; 45], sp1 [44], E2F1 [20], c-myc [46], IRF5 [47], c-Fos [48], NFATc2 [49] and hnRNPk [50]. NF-kB, p53, p63, NFAT, EGR1, hnRNP K, AR and sp1 induce c-FLIP expression, while c-myc, Foxo3a, c-Fos, IRF5 or sp3 inhibit c-FLIP transcription (Figure 2). Activation of these transcription factors can be mediated by a large panel of signaling pathways, including TNF ligands, growth factors, interleukins, chemokines, DNA damaging agents or non-conventional chemotherapeutic agents (Table 1) [51]. Activation of NF-kB by TNFα or CD40 ligand leads to c-FLIP upregulation and to inhibition of Fas-, TNFR1- and TRAIL receptor-induced apoptosis [38; 52; 53]. Likewise,

activation of the PI3K (phosphatidylinositol-3 kinase)/Akt, MAPK (mitogen-activated protein kinase) pathways, or growth factor stimulation, induces the transcriptional upregulation of c-FLIP expression and affords protection to apoptosis induced by death receptors [3]. More recently, the chemokine IL-8 was shown to increase c-FLIP$_S$ and c-FLIP$_L$ mRNA levels through both NF-kB- and androgen-receptor dependent transcriptional activation in prostate cancer cell lines [54]. Alternatively, inhibition of c-FLIP expression has been shown to occur through interferon β-mediated IRF5 activation [47], as well as PMA or TRAIL-induced activation of c-Fos [55; 56].

Regulation of c-FLIP isoforms is still, however, not completely understood, but appears to depend both on the transcription factor itself or the signaling pathway that is activated, and on the specific cell line. For example, in lung cancer cells, E2F1 was shown to inhibit c-FLIP$_S$ expression, but not c-FLIP$_L$[20]. In another study, up-regulation of c-FLIP$_S$ in activated T-cells was shown to specifically rely on NFATc2 [49]. Regulation of the c-FLIP$_R$ isoform is less characterized. CD40-mediated upregulation of c-FLIP$_R$ was demonstrated to inhibit Fas ligand-induced cell death in primary precursor B-ALL [57]. Interestingly, although c-FLIP$_S$ and c-FLIP$_L$ expression are both regulated by NF-kB, c-FLIP$_R$ expression was shown to be induced upon TNF stimulation in a NF-kB-independent manner in the erythroleukemic cell line, TF-1 [58]. Moreover, a RNAi screen aimed at defining p63 targets, in HaCat cells, further highlighted the findings that c-FLIP isoforms may be differentially regulated by a single transcription factor. It was found in this study that p63 could induce specifically c-FLIP$_R$ expression, while repressing that of c-FLIP$_S$ without affecting the transcription levels of c-FLIP$_L$ [41].

The increasing number of transcriptional regulators found to bind CFLAR promoter, thus certainly account for the differential regulation of c-FLIP isoforms, in cell-type dependent manner, and ultimately determine cell fate from a given stimulus [51]. However, the molecular mechanisms that regulate the alternative splicing of the c-FLIP gene, CFLAR, are still

incompletely understood and require further efforts.

4 Post-translational regulation of c-FLIP expression

Besides, transcriptional regulation, c-FLIP isoforms are also heavily regulated at the post-transcriptional level by a plethora of compounds that induce c-FLIP degradation and afford sensitization to death receptor induced apoptosis (Table 1). c-FLIP isoforms are short lived proteins whose expression can easily be attenuated by the use of protein or RNA synthesis inhibitors [11; 59]. Their expression was shown to be regulated by heat stress [60], JNK activation via the E3 ubiquitin ligase ITCH [61] and by the ubiquitin proteasomal pathway [62], through phosphorylation dependent or independent mechanisms (Figure 3).

The pathways for the degradation of c-FLIP by the proteasome are surprisingly complex, partly due to the different mechanisms that exist for the long and the short forms, and to our incomplete understanding of the post-translational modifications that can target c-FLIP isoforms. The short form of c-FLIP is more prone to ubiquitylation and degradation, due to its unique C-terminal tail [5]. The E3 ubiquitin ligase ITCH, which is under control of JNK, builds polyubiquitin chains on c-FLIP to target it for degradation at the proteasome [61]. Initially it was established that only the long form of c-FLIP could be ubiquitylated by ITCH, however more recent work has demonstrated that ITCH is also a key regulator of c-$FLIP_S$ ubiquitylation and stability [63; 64]. Interestingly, ubiquitylation of c-$FLIP_L$ was shown to be independent of CUL3, the E3 ligase that mediates caspase-8 ubiquitylation upon TRAIL stimulation [65]. c-$FLIP_L$ and c-$FLIP_S$ can also be degraded in a JNK-independent manner [66; 67; 68].

Phosphorylation events also play important roles in the regulation of c-FLIP protein levels. Phosphorylation at the serine 193 residue of the c-$FLIP_S$ form inhibits its polyubiquitylation, thus

stabilizing c-$FLIP_S$ levels in the cell [69] and enhancing cell survival. Akt can phosphorylate c-$FLIP_L$ at serine residue 273, which is important in the reduction of c-FLIP levels, though in a JNK and ITCH dependent manner [70]. Conversely, Akt can also enhance the polyubiquitylation and degradation of the E3 ligase ITCH, which acts to stabilize c-$FLIP_S$ levels [63]. Another mechanism has been identified in mouse macrophages undergoing *Mycobacterium*-induced apoptosis, where the protein kinases p38 and c-Abl phosphorylate specific residues on c-$FLIP_S$, facilitating an interaction between c-$FLIP_S$ and the E3 ligase c-Cbl. Ubiquitylation of c-$FLIP_S$ results in its degradation by the proteasome [71]. It may be important to note that the authors in this paper discuss c-$FLIP_S$, rather than c-$FLIP_R$, which is the only short isoform of c-FLIP present in murine cells [49].

Finally, a role for reactive oxygen species (ROS) has also been described for the regulation of c-FLIP, however it is still not clearly understood how this particular mechanism works. ROS were described to induce FLIP downregulation through the proteasome in FasL mediated apoptosis [72]. It has also been demonstrated that NF-κB can inhibit JNK activation by suppressing the levels of ROS in cells [73], which could lead to a decrease in the activity of Itch, thus stabilizing the levels of c-FLIP. This indicates that modulation of NF-κB can affect c-FLIP levels at both the transcriptional and posttranscriptional level.

5 Targeting c-FLIP for cancer therapy

There is a strong correlation between the overexpression of c-FLIP and resistance to FasL- or TRAIL-induced apoptosis, and many *in vitro* studies have demonstrated that inhibiting c-FLIP directly or indirectly can overcome this resistance. Thus targeting c-FLIP may be a promising strategy for cancer therapy, especially if combined with other treatments, such as TRAIL or

conventional chemotherapy [36; 74; 75; 76; 77].

5.1 Transcriptional regulators

Treatment with DNA damaging agents has shown some promise with regard to decreasing c-FLIP levels, however the effect on expression levels varies between cell types. This finding was at first rather unexpected since c-FLIP was shown to be a p53 target [40]. However, the upregulation of c-FLIP by chemotherapeutic drugs has not yet been documented at the protein level. On the contrary, some chemotherapeutic drugs have been shown to downregulate levels of c-FLIP (Table 1.). Cisplatin, in particular, has been well studied in ovarian cancer cell lines, and shown to induce ubiquitylation and degradation of c-FLIP in a p53 dependent manner, through the formation [78]of a ternary complex with both p53 and Itch [79]. Ubiquitylation of c-FLIP$_{S/L}$ by this ternary complex was recently shown to be under the control of the Akt pathway in these ovarian cancer cells [80]. An earlier study also indicated that cisplatin could downregulate c-FLIP$_S$ expression in melanoma cells, but not the long. Instead c-FLIP$_L$ was found to be dephosphorylated upon cisplatin stimulation as evidence by 2D-gel analysis in resistant melanoma cells [78]. Based on earlier studies showing that phosphorylation of c-FLIP$_L$ by CaMKII can promote c-FLIP recruitment and inhibition of caspase-8 within the DISC [81], it was proposed that dephosphorylation of c-FLIP$_L$ would impair its inhibitory activity in glioma cells [78]. In line with the involvement of p53 in regulating c-FLIP expression, oxaliplatin and CPT11 were shown to induce c-FLIP$_{S/L}$ downregulation in the p53 wt colon carcinoma cell line HCT116, but not in the p53-/- isogenic clone [75]. However c-FLIP$_{S/L}$ were also deregulated in the p53 mutated HT29 cell line and sensitization to TRAIL-induced cell death by these drugs was achieved irrespective of the p53 status [75]. Histone deacetylase inhibitors and topoisomerase I inhibitors are two additional emerging cancer therapies which have been shown to regulate c-

FLIP levels. Trichostatin A treatment downregulated c-$FLIP_L$ mRNA and protein levels in ovarian cancer cells, with no effect on c-$FLIP_S$. Interestingly, treatment with inhibitors of the EGRF signaling pathway blocked the regulation of c-$FLIP_L$ by trichostatin A[82]. Two other HDACis, ITF2357 and valproic acid, were studied in heptocellular carcinoma cells. These two agents were also shown to decrease c-FLIP mRNA and protein levels, though the study did not differentiate between the different isoforms [83]. More recently, 4-(4-Chloro-2-methylphenoxy)-N-hydroxybutanamide (CMH), a small molecule inhibitor of c-FLIP, identified using a high-throughput chemical library screen [84; 85], was demonstrated to induce killing in the breast cancer cell line MCF7. CMH induced apoptosis through c-$FLIP_L$ and c-$FLIP_S$ mRNA downregulation [86]. The use of DNA-damaging agents to target c-FLIP or presents some difficulties, as the effect on c-FLIP varies from cell type to cell type, and can affect either both or just one of the isoforms.

5.2 Genetic approaches : siRNAs

Directly inhibiting translation through RNA interference represents the most specific method of downregulating c-FLIP, and this approach has been used in many in vitro studies to sensitize cells to TRAIL or FasL mediated killing [36; 74; 75]. However, there are many limitations for siRNA *in vivo*, and clinical trials using siRNA to target c-FLIP are probably still some time away.

5.3 Targeting c-FLIP for degradation

General metabolic inhibitors were among the first compounds used to study the mechanics of inhibiting c-FLIP expression. c-FLIP has been shown to be downregulated by several compounds that have a broad activity on the cell. For example, cycloheximide [29; 87; 88; 89], or anisomycin

[85], two protein synthesis inhibitors, like the RNA synthesis inhibitor actinomycin D [11; 14; 17; 78], have been shown to downregulate the long and the short forms of c-FLIP (Table 1). Chemotherapy with fluorouracil (5-FU) has also been demonstrated to downregulate both the long and short isoforms of c-FLIP in colon cancer cell lines [75; 90].

The protease inhibitor bortezomib has been widely studied in many different cell lines, with differing outcomes depending on the type of cell line studied. c-FLIP levels in Hodgkins and Reed-Sternberg cells [91], astrocytoma and oligoastrocytoma [92], esthesioneuroblastoma [93], myeloid leukaemia cells [94], and myeloma cell lines [95; 96] were decreased after treatment with bortezomib. While most studies have mainly focused on c-FLIP$_L$, it should be noted however that in at least two studies where the short isoform was analyzed in parallel, c-FLIP$_L$ deregulation was associated with the up-regulation of c-FLIP$_S$, [93; 97]. Likewise, studies on the small molecule proteasome inhibitor MG-132 have also demonstrated both increases and decreases in cellular c-FLIP levels in different cell lines [51]. Strikingly, both bortezomib and MG132 sensitized each of these cell types to TRAIL-induced cell death, indicating that deregulation of both the long and the short isoforms is not an absolute requirement for the restoration of apoptosis. Much effort needs to be made to understand why the remaining c-FLIP isoform fails to protect cells from death ligands.

6 Conclusions

Many in vitro studies have demonstrated the importance of the role of c-FLIP in resistance to apoptosis induced by death receptors and, to a lesser extent, to conventional chemotherapy. Elevated expression of c-FLIP is often identified in malignant cancers, and is strongly correlated with a poor prognosis. Many studies in cell lines demonstrate that sensitivity

to Fas ligand or TRAIL induced apoptosis can be restored by decreasing the levels of c-FLIP in malignant cells. However, as covered in this review, c-FLIP is highly regulated through many different pathways, and the existence of the three separate isoforms, which appear to be differentially modulated, adds another level of complexity with regard to targeting c-FLIP for cancer therapy.

Particularly, it remains unclear whether c-FLIP isoforms may play different regulatory functions in different types of cancer. While most c-FLIP inhibitors described so far target c-$FLIP_L$ and c-$FLIP_S$ isoforms simultaneously, some compounds affect only one isoform. In line with this is the finding that bortezomib can inhibit the expression of c-$FLIP_L$ and induce that of c-$FLIP_S$, but nevertheless can sensitize cells to death receptor induced apoptosis. Post-translational modifications of c-FLIP, including phosphorylation of serine 193, may selectively inactivate one isoform by preventing its recruitment at the DISC level. A better understanding of these post-translational modifications and the systematic analysis of the different isoforms of c-FLIP could in the future help address this question.

Thus far, apart from siRNAs, the inhibitors that have been studied act indirectly on c-FLIP. The development of compounds targeting c-FLIP directly, either at the mRNA or protein level, would be of great interest for further study, but this presents a major challenge. As a key negative regulator of the death receptor apoptotic machinery, c-FLIP represents a promising target for cancer therapy. However c-FLIP is enzymatically inactive, and structurally very similar to caspase-8. Specific targeting of c-FLIP may be difficult and has so far not been achieved. One strategy would be to develop compounds that interfere with the recruitment of c-FLIP to the DISC through the DED domains, however, such compounds would have to be designed so that they would not inhibit recruitment of caspase-8, with its highly homologous DED. Alternatively, some of the inhibitors found so far to target c-FLIP to proteosomal degradation, may in fact act

indirectly at the transcriptional level, and could simultaneously induce the stabilization or degradation of transcription factors found to regulate CFLAR expression. These include p63 which, like c-FLIP, can be targeted to proteosomal degradation by the E3 ligase ITCH [98], or c-myc [99], c-Fos [100] and Foxo3a [101], to name a few. Kinase inhibitors targeting Akt and NF-kB, or multikinase inhibitors such as sorafenib, could also lead to transcriptional and post-transcriptional regulation of c-FLIP expression and function.

The preclinical data clearly indicate that selective inhibitors of c-FLIP, in combination with a ligand such as TRAIL or FasL, or a conventional chemotherapy such as 5-FU, could represent an effective anti-tumor therapy, however, selective inhibition of c-FLIP may be more difficult than anticipated. A deeper understanding of the regulation of the protein, and the role of the different isoforms would be probably required for the rational design or for chemical library screens of selective c-FLIP inhibitors. An ideal situation would be the development of a panel of compounds that can to restore sensitivity to death receptor induced cell death through the specific regulation of the cFLIP isoforms. Using these compounds it could be possible to regulate the levels, or even modulate the ratios of the different isoforms in malignant cells, offering a way in which to control ligand-mediated apoptosis as a therapy for cancer. The future will tell whether such compounds can be obtained.

Conflicts of interest

None declared.

Acknowledgements

We apologize to all authors whose work was not cited in this article due to space limitations. This work is supported by grants of the Conseil Regional de Bourgogne, the INCa (Institut National du Cancer), the Ligue Nationale contre le Cancer, Cancéropôle Grand-Est, ANR (Agence Nationale de la Recherche, ANR-06-JCJC-0103 and 07-PCV-0031) and the European Community (ApopTrain Marie Curie RTN). S.S. is supported by the INCa (POLYNOM-174).

	Primary mode of action	Agent	References
Transcriptional	Alkylating agent	Cisplatin Oxaliplatin	**[75; 78; 79; 102; 103]**
	Intercalating of DNA	Doxorubicin	**[104]**
	Histone deacetylase inhibitor	Vorinostat,Trichostatin, Droxinostat, Valproic acid	**[82; 83; 105; 106]**
	Topoisomerase I inhibitor	Camptothecin, 9-NC, Irinotecan	**[32; 75; 107; 108; 109]**
	Modulation of Ras/PI3K/NF-κB pathway	Lupeol (triterpene)	**[110]**
	Suppression NF-κB pathway	Celastrol, Zerumbone (sesquiterpene), Withaferin A (steroidal lactone) Quinacrine	**[111; 112] [113; 114]**
	Decreases TNFα mediated NF-κB activation	Chrysin (flavanoid)	**[115]**
	Inhibition of STAT3 activation	CDDO-Imidazolide – synthetic triterpenoid	**[116]**
Postranscriptional	RNA interference	siRNAs	**[36; 117]**
	? – phosphorylation of long form	Cisplatin	**[78]**
	RNA synthesis inhibitor	Actinomycin D	**[11; 14; 17]**
	Protein synthesis inhibitor	Cycloheximide Anisomycin	**[29; 85; 87; 88; 89; 118]**
	Thymidylate synthase inhibitor	Fluorouracil (5-FU)	**[75; 90]**
	Proteasome inhibitor	PS-34 (bortezomib)	**[91; 94; 95; 96; 97]**
	Small molecule proteasome inhibitor	MG-132	**[48; 119]**
	PPARγ modulating agent	Troglitazone	**[120; 121; 122]**
	Multikinase inhibitor	Sorafenib	**[123; 124]**
	Antimicrotubule agent	Taxol (paclitaxel), Nocodazole	**[66; 125]**
	Downregulation Akt and NF-κB	Genistein (isoflavone)	**[126]**
	?	Silibinin (Flavonoid)	**[67]**
	COX-2 inhibitor	Celecoxib	**[127]**
	?	CDDO-Me	**[68]**

Table 1. Inhibitors of c-FLIP and their main modes of action.

Figure 1. c-FLIP forms and interaction at the DISC. ***A*** c-FLIP is expressed as three different isoforms in the human cell. c-FLIP$_L$ contains a caspase-like domain. The two aspartate proposed cleavage sites are indicated. c-FLIP$_R$ contains a unique sequence at the C-terminus. ***B*** Interaction of the long and the short c-FLIP isoforms at the DISC. **1-** In the absence of c-FLIP, procaspase-8 dimerization induce full processing and activation of caspase-8, leading to the release of active caspase-8 to the cytosol and activation of apoptosis. **2-** In the presence of c-FLIP$_S$ procaspase-8 remains mostly uncleaved and thus non functional. **3-** When c-FLIP$_L$ is present, procaspase-8 forms heterodimers with c-FLIP$_L$ limiting procaspase-8 autoprocessing. Autoprocessing occurs either between the prodomain and the caspase domain or between the p20 and p10 subunits of the caspase domain. The active heterodimer remains associated with the DISC complex where it can cleave a limited number of substrates including RIP leading to enforced NF-kB activation of the activation of non apoptotic signaling pathways such as ERK. Like c-FLIP$_S$, c-FLIP$_L$ prevents death-receptor induced apoptosis.

Figure 2. Signaling pathways leading to the upregulation or downregulation of c-FLIP. See text for details.

Figure 3. Posttranslation modifications and regulation of degradation of c-FLIP. JNK activates the E3 ubiquitin ligase ITCH to mediate accelerated turnover of c-FLIP$_L$ and c-FLIP$_S$ through polyubiquitylation and degradation by the proteasome. Different compounds have been identified which accelerate the degradation of either c-FLIP$_L$, c-FLIP$_S$. Phosphorylation events by p38 and c-Abl allow polyubiquitylation of c-FLIP$_S$ by c-Cbl, resulting in degradation. Akt phosphorylates the c-FLIP$_L$ form, enhancing an ITCH independent degradation of the protein. Conversely, loss of PTEN and activation of Akt are events in a pathway that bring about the ubiquitylation of ITCH, which results in stabilization of c-FLIP$_S$. Protein kinase C (PKC) has been shown to phosphorylate c-FLIP$_S$ at a specific serine, decreasing its ubiquitylation and stabilizing levels of c-FLIP$_S$. Finally, generation of ROS, can also enhance degradation of cFLIP.

[1] M.A. Hughes, N. Harper, M. Butterworth, K. Cain, G.M. Cohen, M. MacFarlane, Reconstitution of the death-inducing signaling complex reveals a substrate switch that determines CD95-mediated death or survival. Molecular cell 35 (2009) 265-279.
[2] O. Micheau, J. Tschopp, Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. Cell 114 (2003) 181-190.
[3] O. Micheau, Cellular FLICE-inhibitory protein: an attractive therapeutic target? Expert opinion on therapeutic targets 7 (2003) 559-573.
[4] M. Irmler, M. Thome, M. Hahne, P. Schneider, K. Hofmann, V. Steiner, J.L. Bodmer, M. Schroter, K. Burns, C. Mattmann, D. Rimoldi, L.E. French, J. Tschopp, Inhibition of death receptor signals by cellular FLIP. Nature 388 (1997) 190-195.
[5] M. Poukkula, A. Kaunisto, V. Hietakangas, K. Denessiouk, T. Katajamaki, M.S. Johnson, L. Sistonen, J.E. Eriksson, Rapid turnover of c-FLIPshort is determined by its unique C-terminal tail. The Journal of biological chemistry 280 (2005) 27345-27355.
[6] N. Ueffing, E. Keil, C. Freund, R. Kuhne, K. Schulze-Osthoff, I. Schmitz, Mutational analyses of c-FLIPR, the only murine short FLIP isoform, reveal requirements for DISC recruitment. Cell Death Differ 15 (2008) 773-782.
[7] O. Micheau, M. Thome, P. Schneider, N. Holler, J. Tschopp, D.W. Nicholson, C. Briand, M.G. Grutter, The long form of FLIP is an activator of caspase-8 at the Fas death-inducing signaling complex. The Journal of biological chemistry 277 (2002) 45162-45171.
[8] H. Wajant, E. Haas, R. Schwenzer, F. Muhlenbeck, S. Kreuz, G. Schubert, M. Grell, C. Smith, P. Scheurich, Inhibition of death receptor-mediated gene induction by a cycloheximide-sensitive factor occurs at the level of or upstream of Fas-associated death domain protein (FADD). The Journal of biological chemistry 275 (2000) 24357-24366.
[9] C. Scaffidi, I. Schmitz, P.H. Krammer, M.E. Peter, The role of c-FLIP in modulation of CD95-induced apoptosis. The Journal of biological chemistry 274 (1999) 1541-1548.
[10] D.W. Chang, Z. Xing, Y. Pan, A. Algeciras-Schimnich, B.C. Barnhart, S. Yaish-Ohad, M.E. Peter, X. Yang, c-FLIP(L) is a dual function regulator for caspase-8 activation and CD95-mediated apoptosis. Embo J 21 (2002) 3704-3714.
[11] A. Hernandez, Q.D. Wang, S.A. Schwartz, B.M. Evers, Sensitization of human colon cancer cells to TRAIL-mediated apoptosis. J Gastrointest Surg 5 (2001) 56-65.
[12] S.Y. Nam, G.A. Jung, G.C. Hur, H.Y. Chung, W.H. Kim, D.W. Seol, B.L. Lee, Upregulation of FLIP(S) by Akt, a possible inhibition mechanism of TRAIL-induced apoptosis in human gastric cancers. Cancer Sci 94 (2003) 1066-1073.
[13] A. Elnemr, T. Ohta, A. Yachie, M. Kayahara, H. Kitagawa, T. Fujimura, I. Ninomiya, S. Fushida, G.I. Nishimura, K. Shimizu, K. Miwa, Human pancreatic cancer cells disable function of Fas receptors at several levels in Fas signal transduction pathway. Int J Oncol 18 (2001) 311-316.
[14] T.S. Griffith, W.A. Chin, G.C. Jackson, D.H. Lynch, M.Z. Kubin, Intracellular regulation of TRAIL-induced apoptosis in human melanoma cells. J Immunol 161 (1998) 2833-2840.
[15] C.W. Xiao, X. Yan, Y. Li, S.A. Reddy, B.K. Tsang, Resistance of human ovarian cancer cells to tumor necrosis factor alpha is a consequence of nuclear factor kappaB-mediated induction of Fas-associated death domain-like interleukin-1beta-converting enzyme-like inhibitory protein. Endocrinology 144 (2003) 623-630.

[16] X. Zhang, T.G. Jin, H. Yang, W.C. DeWolf, R. Khosravi-Far, A.F. Olumi, Persistent c-FLIP(L) expression is necessary and sufficient to maintain resistance to tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis in prostate cancer. Cancer research 64 (2004) 7086-7091.
[17] A. Olsson, T. Diaz, M. Aguilar-Santelises, A. Osterborg, F. Celsing, M. Jondal, L.M. Osorio, Sensitization to TRAIL-induced apoptosis and modulation of FLICE-inhibitory protein in B chronic lymphocytic leukemia by actinomycin D. Leukemia 15 (2001) 1868-1877.
[18] M. MacFarlane, N. Harper, R.T. Snowden, M.J. Dyer, G.A. Barnett, J.H. Pringle, G.M. Cohen, Mechanisms of resistance to TRAIL-induced apoptosis in primary B cell chronic lymphocytic leukaemia. Oncogene 21 (2002) 6809-6818.
[19] P. Korkolopoulou, A. Goudopoulou, G. Voutsinas, E. Thomas-Tsagli, P. Kapralos, E. Patsouris, A.A. Saetta, c-FLIP expression in bladder urothelial carcinomas: its role in resistance to Fas-mediated apoptosis and clinicopathologic correlations. Urology 63 (2004) 1198-1204.
[20] C. Salon, B. Eymin, O. Micheau, L. Chaperot, J. Plumas, C. Brambilla, E. Brambilla, S. Gazzeri, E2F1 induces apoptosis and sensitizes human lung adenocarcinoma cells to death-receptor-mediated apoptosis through specific downregulation of c-FLIP(short). Cell Death Differ 13 (2006) 260-272.
[21] H. Zong, B. Yin, J. Chen, B. Ma, D. Cai, X. He, Over-expression of c-FLIP confers the resistance to TRAIL-induced apoptosis on gallbladder carcinoma. Tohoku J Exp Med 217 (2009) 203-208.
[22] H. Okano, K. Shiraki, H. Inoue, T. Kawakita, T. Yamanaka, M. Deguchi, K. Sugimoto, T. Sakai, S. Ohmori, K. Fujikawa, K. Murata, T. Nakano, Cellular FLICE/caspase-8-inhibitory protein as a principal regulator of cell death and survival in human hepatocellular carcinoma. Laboratory investigation; a journal of technical methods and pathology 83 (2003) 1033-1043.
[23] S.H. Lee, H.S. Kim, S.Y. Kim, Y.S. Lee, W.S. Park, S.H. Kim, J.Y. Lee, N.J. Yoo, Increased expression of FLIP, an inhibitor of Fas-mediated apoptosis, in stomach cancer. Apmis 111 (2003) 309-314.
[24] X.D. Zhou, J.P. Yu, J. Liu, H.S. Luo, H.X. Chen, H.G. Yu, Overexpression of cellular FLICE-inhibitory protein (FLIP) in gastric adenocarcinoma. Clin Sci (Lond) 106 (2004) 397-405.
[25] S. Mathas, A. Lietz, I. Anagnostopoulos, F. Hummel, B. Wiesner, M. Janz, F. Jundt, B. Hirsch, K. Johrens-Leder, H.P. Vornlocher, K. Bommert, H. Stein, B. Dorken, c-FLIP mediates resistance of Hodgkin/Reed-Sternberg cells to death receptor-induced apoptosis. J Exp Med 199 (2004) 1041-1052.
[26] N.S. Wilson, V. Dixit, A. Ashkenazi, Death receptor signal transducers: nodes of coordination in immune signaling networks. Nat Immunol 10 (2009) 348-355.
[27] R.R. Bullani, B. Huard, I. Viard-Leveugle, H.R. Byers, M. Irmler, J.H. Saurat, J. Tschopp, L.E. French, Selective expression of FLIP in malignant melanocytic skin lesions. J Invest Dermatol 117 (2001) 360-364.
[28] A.S. de Hooge, D. Berghuis, S.J. Santos, E. Mooiman, S. Romeo, J.A. Kummer, R.M. Egeler, M.J. van Tol, C.J. Melief, P.C. Hogendoorn, A.C. Lankester, Expression of cellular FLICE inhibitory protein, caspase-8, and protease inhibitor-9 in Ewing sarcoma and implications for susceptibility to cytotoxic pathways. Clin Cancer Res 13 (2007) 206-214.
[29] T. Mori, R. Doi, E. Toyoda, M. Koizumi, D. Ito, K. Kami, A. Kida, T. Masui, Y. Kawaguchi, K. Fujimoto, Regulation of the resistance to TRAIL-induced apoptosis as a new strategy for pancreatic cancer. Surgery 138 (2005) 71-77.

[30] M. Djerbi, V. Screpanti, A.I. Catrina, B. Bogen, P. Biberfeld, A. Grandien, The inhibitor of death receptor signaling, FLICE-inhibitory protein defines a new class of tumor progression factors. J Exp Med 190 (1999) 1025-1032.
[31] G.J. Ullenhag, A. Mukherjee, N.F. Watson, A.H. Al-Attar, J.H. Scholefield, L.G. Durrant, Overexpression of FLIPL is an independent marker of poor prognosis in colorectal cancer patients. Clin Cancer Res 13 (2007) 5070-5075.
[32] W. Wang, S. Wang, X. Song, N. Sima, X. Xu, A. Luo, G. Chen, D. Deng, Q. Xu, L. Meng, Y. Lu, D. Ma, The relationship between c-FLIP expression and human papillomavirus E2 gene disruption in cervical carcinogenesis. Gynecol Oncol 105 (2007) 571-577.
[33] M.B. Valnet-Rabier, B. Challier, S. Thiebault, R. Angonin, G. Margueritte, C. Mougin, B. Kantelip, E. Deconinck, J.Y. Cahn, T. Fest, c-Flip protein expression in Burkitt's lymphomas is associated with a poor clinical outcome. Br J Haematol 128 (2005) 767-773.
[34] G. Valente, F. Manfroi, C. Peracchio, G. Nicotra, R. Castino, G. Nicosia, S. Kerim, C. Isidoro, cFLIP expression correlates with tumour progression and patient outcome in non-Hodgkin lymphomas of low grade of malignancy. Br J Haematol 132 (2006) 560-570.
[35] E.W. Duiker, A.G. van der Zee, P. de Graeff, W. Boersma-van Ek, H. Hollema, G.H. de Bock, S. de Jong, E.G. de Vries, The extrinsic apoptosis pathway and its prognostic impact in ovarian cancer. Gynecol Oncol 116 (2010) 549-555.
[36] D.B. Longley, T.R. Wilson, M. McEwan, W.L. Allen, U. McDermott, L. Galligan, P.G. Johnston, c-FLIP inhibits chemotherapy-induced colorectal cancer cell death. Oncogene 25 (2006) 838-848.
[37] O. Micheau, E. Solary, A. Hammann, M.T. Dimanche-Boitrel, Fas ligand-independent, FADD-mediated activation of the Fas death pathway by anticancer drugs. The Journal of biological chemistry 274 (1999) 7987-7992.
[38] O. Micheau, S. Lens, O. Gaide, K. Alevizopoulos, J. Tschopp, NF-kappaB signals induce the expression of c-FLIP. Mol Cell Biol 21 (2001) 5299-5305.
[39] S. Kreuz, D. Siegmund, P. Scheurich, H. Wajant, NF-kappaB inducers upregulate cFLIP, a cycloheximide-sensitive inhibitor of death receptor signaling. Mol Cell Biol 21 (2001) 3964-3973.
[40] T. Bartke, D. Siegmund, N. Peters, M. Reichwein, F. Henkler, P. Scheurich, H. Wajant, p53 upregulates cFLIP, inhibits transcription of NF-kappaB-regulated genes and induces caspase-8-independent cell death in DLD-1 cells. Oncogene 20 (2001) 571-580.
[41] S. Borrelli, E. Candi, D. Alotto, C. Castagnoli, G. Melino, M.A. Vigano, R. Mantovani, p63 regulates the caspase-8-FLIP apoptotic pathway in epidermis. Cell Death Differ 16 (2009) 253-263.
[42] C. Skurk, H. Maatz, H.S. Kim, J. Yang, M.R. Abid, W.C. Aird, K. Walsh, The Akt-regulated forkhead transcription factor FOXO3a controls endothelial cell viability through modulation of the caspase-8 inhibitor FLIP. J Biol Chem 279 (2004) 1513-1525.
[43] D. Mahalingam, A. Natoni, M. Keane, A. Samali, E. Szegezdi, Early growth response-1 is a regulator of DR5-induced apoptosis in colon cancer cells. Br J Cancer 102 (2010) 754-764.
[44] M. Ganapathy, R. Ghosh, X. Jianping, X. Zhang, R. Bedolla, J. Schoolfield, I.T. Yeh, D.A. Troyer, A.F. Olumi, A.P. Kumar, Involvement of FLIP in 2-methoxyestradiol-induced tumor regression in transgenic adenocarcinoma of mouse prostate model. Clin Cancer Res 15 (2009) 1601-1611.
[45] S. Gao, H. Wang, P. Lee, J. Melamed, C.X. Li, F. Zhang, H. Wu, L. Zhou, Z. Wang, Androgen receptor and prostate apoptosis response factor-4 target the c-FLIP gene to determine survival and apoptosis in the prostate gland. J Mol Endocrinol 36 (2006) 463-483.

[46] M.S. Ricci, Z. Jin, M. Dews, D. Yu, A. Thomas-Tikhonenko, D.T. Dicker, W.S. El-Deiry, Direct repression of FLIP expression by c-myc is a major determinant of TRAIL sensitivity. Mol Cell Biol 24 (2004) 8541-8555.
[47] G. Hu, B.J. Barnes, IRF-5 is a mediator of the death receptor-induced apoptotic signaling pathway. The Journal of biological chemistry 284 (2009) 2767-2777.
[48] W. Li, X. Zhang, A.F. Olumi, MG-132 sensitizes TRAIL-resistant prostate cancer cells by activating c-Fos/c-Jun heterodimers and repressing c-FLIP(L). Cancer research 67 (2007) 2247-2255.
[49] N. Ueffing, M. Schuster, E. Keil, K. Schulze-Osthoff, I. Schmitz, Up-regulation of c-FLIP short by NFAT contributes to apoptosis resistance of short-term activated T cells. Blood 112 (2008) 690-698.
[50] L.C. Chen, I.C. Chung, C. Hsueh, N.M. Tsang, L.M. Chi, Y. Liang, C.C. Chen, L.J. Wang, Y.S. Chang, The antiapoptotic protein, FLIP, is regulated by heterogeneous nuclear ribonucleoprotein K and correlates with poor overall survival of nasopharyngeal carcinoma patients. Cell Death Differ (2010).
[51] O. Micheau, Cellular FLICE-inhibitory Protein: An Update, in: P.D.D.S.F. Prof. Dr. Klaus-Michael Debatin, (Ed.), Apoptosis and Cancer Therapy, 2008, pp. 120-156.
[52] A. Hennino, M. Berard, M. Casamayor-Palleja, P.H. Krammer, T. Defrance, Regulation of the Fas death pathway by FLICE-inhibitory protein in primary human B cells. J Immunol 165 (2000) 3023-3030.
[53] M. Travert, P. Ame-Thomas, C. Pangault, A. Morizot, O. Micheau, G. Semana, T. Lamy, T. Fest, K. Tarte, T. Guillaudeux, CD40 ligand protects from TRAIL-induced apoptosis in follicular lymphomas through NF-kappaB activation and up-regulation of c-FLIP and Bcl-xL. J Immunol 181 (2008) 1001-1011.
[54] C. Wilson, T. Wilson, P.G. Johnston, D.B. Longley, D.J. Waugh, Interleukin-8 signaling attenuates TRAIL- and chemotherapy-induced apoptosis through transcriptional regulation of c-FLIP in prostate cancer cells. Mol Cancer Ther 7 (2008) 2649-2661.
[55] X. Zhang, W. Li, A.F. Olumi, Low-dose 12-O-tetradecanoylphorbol-13-acetate enhances tumor necrosis factor related apoptosis-inducing ligand induced apoptosis in prostate cancer cells. Clin Cancer Res 13 (2007) 7181-7190.
[56] X. Zhang, L. Zhang, H. Yang, X. Huang, H. Otu, T.A. Libermann, W.C. DeWolf, R. Khosravi-Far, A.F. Olumi, c-Fos as a proapoptotic agent in TRAIL-induced apoptosis in prostate cancer cells. Cancer research 67 (2007) 9425-9434.
[57] A. Troeger, I. Schmitz, M. Siepermann, L. Glouchkova, U. Gerdemann, G.E. Janka-Schaub, K. Schulze-Osthoff, D. Dilloo, Up-regulation of c-FLIPS+R upon CD40 stimulation is associated with inhibition of CD95-induced apoptosis in primary precursor B-ALL. Blood 110 (2007) 384-387.
[58] S.A. Rushworth, A. Taylor, S. Langa, D.J. MacEwan, TNF signaling gets FLIPped off: TNF-induced regulation of FLIP. Cell cycle (Georgetown, Tex 7 (2008) 194-199.
[59] S. Fulda, E. Meyer, K.M. Debatin, Metabolic inhibitors sensitize for CD95 (APO-1/Fas)-induced apoptosis by down-regulating Fas-associated death domain-like interleukin 1-converting enzyme inhibitory protein expression. Cancer research 60 (2000) 3947-3956.
[60] S.E. Tran, A. Meinander, T.H. Holmstrom, A. Rivero-Muller, K.M. Heiskanen, E.K. Linnau, M.J. Courtney, D.D. Mosser, L. Sistonen, J.E. Eriksson, Heat stress downregulates FLIP and sensitizes cells to Fas receptor-mediated apoptosis. Cell Death Differ 10 (2003) 1137-1147.

[61] L. Chang, H. Kamata, G. Solinas, J.L. Luo, S. Maeda, K. Venuprasad, Y.C. Liu, M. Karin, The E3 ubiquitin ligase itch couples JNK activation to TNFalpha-induced cell death by inducing c-FLIP(L) turnover. Cell 124 (2006) 601-613.
[62] T. Fukazawa, T. Fujiwara, F. Uno, F. Teraishi, Y. Kadowaki, T. Itoshima, Y. Takata, S. Kagawa, J.A. Roth, J. Tschopp, N. Tanaka, Accelerated degradation of cellular FLIP protein through the ubiquitin- proteasome pathway in p53-mediated apoptosis of human cancer cells. Oncogene 20 (2001) 5225-5231.
[63] A. Panner, C.A. Crane, C. Weng, A. Feletti, A.T. Parsa, R.O. Pieper, A novel PTEN-dependent link to ubiquitination controls FLIPS stability and TRAIL sensitivity in glioblastoma multiforme. Cancer research 69 (2009) 7911-7916.
[64] A. Panner, C.A. Crane, C. Weng, A. Feletti, S. Fang, A.T. Parsa, R.O. Pieper, Ubiquitin-specific protease 8 links the PTEN-Akt-AIP4 pathway to the control of FLIPS stability and TRAIL sensitivity in glioblastoma multiforme. Cancer research 70 (2010) 5046-5053.
[65] Z. Jin, Y. Li, R. Pitti, D. Lawrence, V.C. Pham, J.R. Lill, A. Ashkenazi, Cullin3-based polyubiquitination and p62-dependent aggregation of caspase-8 mediate extrinsic apoptosis signaling. Cell 137 (2009) 721-735.
[66] T. Sanchez-Perez, G. Ortiz-Ferron, A. Lopez-Rivas, Mitotic arrest and JNK-induced proteasomal degradation of FLIP and Mcl-1 are key events in the sensitization of breast tumor cells to TRAIL by antimicrotubule agents. Cell Death Differ 17 (2010) 883-894.
[67] Y.G. Son, E.H. Kim, J.Y. Kim, S.U. Kim, T.K. Kwon, A.R. Yoon, C.O. Yun, K.S. Choi, Silibinin sensitizes human glioma cells to TRAIL-mediated apoptosis via DR5 up-regulation and down-regulation of c-FLIP and survivin. Cancer research 67 (2007) 8274-8284.
[68] W. Zou, S. Chen, X. Liu, P. Yue, M.B. Sporn, F.R. Khuri, S.Y. Sun, c-FLIP downregulation contributes to apoptosis induction by the novel synthetic triterpenoid methyl-2-cyano-3, 12-dioxooleana-1, 9-dien-28-oate (CDDO-Me) in human lung cancer cells. Cancer Biol Ther 6 (2007) 1614-1620.
[69] A. Kaunisto, V. Kochin, T. Asaoka, A. Mikhailov, M. Poukkula, A. Meinander, J.E. Eriksson, PKC-mediated phosphorylation regulates c-FLIP ubiquitylation and stability. Cell Death Differ 16 (2009) 1215-1226.
[70] B. Shi, T. Tran, R. Sobkoviak, R.M. Pope, Activation-induced degradation of FLIP(L) is mediated via the phosphatidylinositol 3-kinase/Akt signaling pathway in macrophages. The Journal of biological chemistry 284 (2009) 14513-14523.
[71] M. Kundu, S.K. Pathak, K. Kumawat, S. Basu, G. Chatterjee, S. Pathak, T. Noguchi, K. Takeda, H. Ichijo, C.B. Thien, W.Y. Langdon, J. Basu, A TNF- and c-Cbl-dependent FLIP(S)-degradation pathway and its function in Mycobacterium tuberculosis-induced macrophage apoptosis. Nat Immunol 10 (2009) 918-926.
[72] L. Wang, N. Azad, L. Kongkaneramit, F. Chen, Y. Lu, B.H. Jiang, Y. Rojanasakul, The Fas death signaling pathway connecting reactive oxygen species generation and FLICE inhibitory protein down-regulation. J Immunol 180 (2008) 3072-3080.
[73] H. Nakano, A. Nakajima, S. Sakon-Komazawa, J.H. Piao, X. Xue, K. Okumura, Reactive oxygen species mediate crosstalk between NF-kappaB and JNK. Cell Death Differ 13 (2006) 730-737.
[74] M. Chawla-Sarkar, S.I. Bae, F.J. Reu, B.S. Jacobs, D.J. Lindner, E.C. Borden, Downregulation of Bcl-2, FLIP or IAPs (XIAP and survivin) by siRNAs sensitizes resistant melanoma cells to Apo2L/TRAIL-induced apoptosis. Cell Death Differ 11 (2004) 915-923.

[75] L. Galligan, D.B. Longley, M. McEwan, T.R. Wilson, K. McLaughlin, P.G. Johnston, Chemotherapy and TRAIL-mediated colon cancer cell death: the roles of p53, TRAIL receptors, and c-FLIP. Mol Cancer Ther 4 (2005) 2026-2036.
[76] M. Bagnoli, S. Canevari, D. Mezzanzanica, Cellular FLICE-inhibitory protein (c-FLIP) signalling: a key regulator of receptor-mediated apoptosis in physiologic context and in cancer. Int J Biochem Cell Biol 42 (2010) 210-213.
[77] A.R. Safa, T.W. Day, C.H. Wu, Cellular FLICE-like inhibitory protein (C-FLIP): a novel target for cancer therapy. Curr Cancer Drug Targets 8 (2008) 37-46.
[78] J.H. Song, D.K. Song, M. Herlyn, K.C. Petruk, C. Hao, Cisplatin down-regulation of cellular Fas-associated death domain-like interleukin-1beta-converting enzyme-like inhibitory proteins to restore tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis in human melanoma cells. Clin Cancer Res 9 (2003) 4255-4266.
[79] M.R. Abedini, E.J. Muller, J. Brun, R. Bergeron, D.A. Gray, B.K. Tsang, Cisplatin induces p53-dependent FLICE-like inhibitory protein ubiquitination in ovarian cancer cells. Cancer research 68 (2008) 4511-4517.
[80] M.R. Abedini, E.J. Muller, R. Bergeron, D.A. Gray, B.K. Tsang, Akt promotes chemoresistance in human ovarian cancer cells by modulating cisplatin-induced, p53-dependent ubiquitination of FLICE-like inhibitory protein. Oncogene 29 (2010) 11-25.
[81] B.F. Yang, C. Xiao, W.H. Roa, P.H. Krammer, C. Hao, Calcium/calmodulin-dependent protein kinase II regulation of c-FLIP expression and phosphorylation in modulation of Fas-mediated signaling in malignant glioma cells. The Journal of biological chemistry 278 (2003) 7043-7050.
[82] S.J. Park, M.J. Kim, H.B. Kim, H.Y. Sohn, J.H. Bae, C.D. Kang, S.H. Kim, Trichostatin A sensitizes human ovarian cancer cells to TRAIL-induced apoptosis by down-regulation of c-FLIPL via inhibition of EGFR pathway. Biochem Pharmacol 77 (2009) 1328-1336.
[83] A. Pathil, S. Armeanu, S. Venturelli, P. Mascagni, T.S. Weiss, M. Gregor, U.M. Lauer, M. Bitzer, HDAC inhibitor treatment of hepatoma cells induces both TRAIL-independent apoptosis and restoration of sensitivity to TRAIL. Hepatology (Baltimore, Md 43 (2006) 425-434.
[84] A.D. Schimmer, M.P. Thomas, R. Hurren, M. Gronda, M. Pellecchia, G.R. Pond, M. Konopleva, D. Gurfinkel, I.A. Mawji, E. Brown, J.C. Reed, Identification of small molecules that sensitize resistant tumor cells to tumor necrosis factor-family death receptors. Cancer research 66 (2006) 2367-2375.
[85] I.A. Mawji, C.D. Simpson, M. Gronda, M.A. Williams, R. Hurren, C.J. Henderson, A. Datti, J.L. Wrana, A.D. Schimmer, A chemical screen identifies anisomycin as an anoikis sensitizer that functions by decreasing FLIP protein synthesis. Cancer research 67 (2007) 8307-8315.
[86] K. Bijangi-Vishehsaraei, M.R. Saadatzadeh, S. Huang, M.P. Murphy, A.R. Safa, 4-(4-Chloro-2-methylphenoxy)-N-hydroxybutanamide (CMH) targets mRNA of the c-FLIP variants and induces apoptosis in MCF-7 human breast cancer cells. Mol Cell Biochem 342 (2010) 133-142.
[87] L.E. Perez, N. Parquet, K. Shain, R. Nimmanapalli, M. Alsina, C. Anasetti, W. Dalton, Bone marrow stroma confers resistance to Apo2 ligand/TRAIL in multiple myeloma in part by regulating c-FLIP. J Immunol 180 (2008) 1545-1555.
[88] D. Mezzanzanica, E. Balladore, F. Turatti, E. Luison, P. Alberti, M. Bagnoli, M. Figini, A. Mazzoni, F. Raspagliesi, M. Oggionni, S. Pilotti, S. Canevari, CD95-mediated apoptosis is impaired at receptor level by cellular FLICE-inhibitory protein (long form) in wild-type p53 human ovarian carcinoma. Clin Cancer Res 10 (2004) 5202-5214.

[89] R.K. Thomas, A. Kallenborn, C. Wickenhauser, J.L. Schultze, A. Draube, M. Vockerodt, D. Re, V. Diehl, J. Wolf, Constitutive expression of c-FLIP in Hodgkin and Reed-Sternberg cells. Am J Pathol 160 (2002) 1521-1528.
[90] S. Lacour, O. Micheau, A. Hammann, V. Drouineaud, J. Tschopp, E. Solary, M.T. Dimanche-Boitrel, Chemotherapy enhances TNF-related apoptosis-inducing ligand DISC assembly in HT29 human colon cancer cells. Oncogene 22 (2003) 1807-1816.
[91] X. Zhao, W. Qiu, J. Kung, X. Peng, M. Yegappan, B. Yen-Lieberman, E.D. Hsi, Bortezomib induces caspase-dependent apoptosis in Hodgkin lymphoma cell lines and is associated with reduced c-FLIP expression: a gene expression profiling study with implications for potential combination therapies. Leukemia research 32 (2008) 275-285.
[92] R. Koschny, H. Holland, J. Sykora, T.L. Haas, M.R. Sprick, T.M. Ganten, W. Krupp, M. Bauer, P. Ahnert, J. Meixensberger, H. Walczak, Bortezomib Sensitizes Primary Human Astrocytoma Cells of WHO Grades I to IV for Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand-Induced Apoptosis. Clin Cancer Res 13 (2007) 3403-3412.
[93] R. Koschny, H. Holland, J. Sykora, H. Erdal, W. Krupp, M. Bauer, U. Bockmuehl, P. Ahnert, J. Meixensberger, W. Stremmel, H. Walczak, T.M. Ganten, Bortezomib sensitizes primary human esthesioneuroblastoma cells to TRAIL-induced apoptosis. J Neurooncol 97 (2010) 171-185.
[94] R. Riccioni, M. Senese, D. Diverio, V. Riti, S. Buffolino, G. Mariani, A. Boe, M. Cedrone, F. Lo-Coco, R. Foa, C. Peschle, U. Testa, M4 and M5 acute myeloid leukaemias display a high sensitivity to Bortezomib-mediated apoptosis. Br J Haematol 139 (2007) 194-205.
[95] L.E. Perez, N. Parquet, M. Meads, C. Anasetti, W. Dalton, Bortezomib restores stroma-mediated APO2L/TRAIL apoptosis resistance in multiple myeloma. Eur J Haematol 84 (2010) 212-222.
[96] P. Balsas, N. Lopez-Royuela, P. Galan-Malo, A. Anel, I. Marzo, J. Naval, Cooperation between Apo2L/TRAIL and bortezomib in multiple myeloma apoptosis. Biochem Pharmacol 77 (2009) 804-812.
[97] X. Liu, P. Yue, S. Chen, L. Hu, S. Lonial, F.R. Khuri, S.Y. Sun, The proteasome inhibitor PS-341 (bortezomib) up-regulates DR5 expression leading to induction of apoptosis and enhancement of TRAIL-induced apoptosis despite up-regulation of c-FLIP and survivin expression in human NSCLC cells. Cancer research 67 (2007) 4981-4988.
[98] G. Melino, R.A. Knight, G. Cesareni, Degradation of p63 by Itch. Cell cycle (Georgetown, Tex 5 (2006) 1735-1739.
[99] M.A. Gregory, S.R. Hann, c-Myc proteolysis by the ubiquitin-proteasome pathway: stabilization of c-Myc in Burkitt's lymphoma cells. Mol Cell Biol 20 (2000) 2423-2435.
[100] C. Tsurumi, N. Ishida, T. Tamura, A. Kakizuka, E. Nishida, E. Okumura, T. Kishimoto, M. Inagaki, K. Okazaki, N. Sagata, et al., Degradation of c-Fos by the 26S proteasome is accelerated by c-Jun and multiple protein kinases. Mol Cell Biol 15 (1995) 5682-5687.
[101] D.R. Plas, C.B. Thompson, Akt activation promotes degradation of tuberin and FOXO3a via the proteasome. The Journal of biological chemistry 278 (2003) 12361-12366.
[102] H. Kinoshita, H. Yoshikawa, K. Shiiki, Y. Hamada, Y. Nakajima, K. Tasaka, Cisplatin (CDDP) sensitizes human osteosarcoma cell to Fas/CD95-mediated apoptosis by down-regulating FLIP-L expression. Int J Cancer 88 (2000) 986-991.
[103] S.J. White-Gilbertson, L. Kasman, J. McKillop, T. Tirodkar, P. Lu, C. Voelkel-Johnson, Oxidative stress sensitizes bladder cancer cells to TRAIL mediated apoptosis by down-regulating anti-apoptotic proteins. J Urol 182 (2009) 1178-1185.

[104] A. El-Zawahry, J. McKillop, C. Voelkel-Johnson, Doxorubicin increases the effectiveness of Apo2L/TRAIL for tumor growth inhibition of prostate cancer xenografts. BMC Cancer 5 (2005) 2.
[105] G. Zhang, M.A. Park, C. Mitchell, H. Hamed, M. Rahmani, A.P. Martin, D.T. Curiel, A. Yacoub, M. Graf, R. Lee, J.D. Roberts, P.B. Fisher, S. Grant, P. Dent, Vorinostat and sorafenib synergistically kill tumor cells via FLIP suppression and CD95 activation. Clin Cancer Res 14 (2008) 5385-5399.
[106] T.E. Wood, S. Dalili, C.D. Simpson, M.A. Sukhai, R. Hurren, K. Anyiwe, X. Mao, F. Suarez Saiz, M. Gronda, Y. Eberhard, N. MacLean, T. Ketela, J.C. Reed, J. Moffat, M.D. Minden, R.A. Batey, A.D. Schimmer, Selective inhibition of histone deacetylases sensitizes malignant cells to death receptor ligands. Mol Cancer Ther 9 (2010) 246-256.
[107] P. Wang, J. Zhang, A. Bellail, W. Jiang, J. Hugh, N.M. Kneteman, C. Hao, Inhibition of RIP and c-FLIP enhances TRAIL-induced apoptosis in pancreatic cancer cells. Cell Signal 19 (2007) 2237-2246.
[108] D. Chatterjee, M. Goldman, C.D. Braastad, J. Darnowski, J.H. Wyche, P. Pantazis, L. Goodglick, Reduction of 9-nitrocamptothecin-triggered apoptosis in DU-145 human prostate cancer cells by ectopic expression of 14-3-3zeta. Int J Oncol 25 (2004) 503-509.
[109] S. Tomek, P. Horak, I. Pribill, G. Haller, M. Rossler, C.C. Zielinski, D. Pils, M. Krainer, Resistance to TRAIL-induced apoptosis in ovarian cancer cell lines is overcome by co-treatment with cytotoxic drugs. Gynecol Oncol 94 (2004) 107-114.
[110] I. Murtaza, M. Saleem, V.M. Adhami, B.B. Hafeez, H. Mukhtar, Suppression of cFLIP by lupeol, a dietary triterpene, is sufficient to overcome resistance to TRAIL-mediated apoptosis in chemoresistant human pancreatic cancer cells. Cancer research 69 (2009) 1156-1165.
[111] B. Sung, B. Park, V.R. Yadav, B.B. Aggarwal, Celastrol, a triterpene, enhances TRAIL-induced apoptosis through the down-regulation of cell survival proteins and up-regulation of death receptors. J Biol Chem 285 (2010) 11498-11507.
[112] Y. Takada, A. Murakami, B.B. Aggarwal, Zerumbone abolishes NF-kappaB and IkappaBalpha kinase activation leading to suppression of antiapoptotic and metastatic gene expression, upregulation of apoptosis, and downregulation of invasion. Oncogene 24 (2005) 6957-6969.
[113] T.J. Lee, H.J. Um, S. Min do, J.W. Park, K.S. Choi, T.K. Kwon, Withaferin A sensitizes TRAIL-induced apoptosis through reactive oxygen species-mediated up-regulation of death receptor 5 and down-regulation of c-FLIP. Free Radic Biol Med 46 (2009) 1639-1649.
[114] T.S. Jani, J. Devecchio, T. Mazumdar, A. Agyeman, J.A. Houghton, Inhibition of NF-{kappa}B signaling by quinacrine is cytotoxic to human colon carcinoma cell lines (cc) and is synergistic in combination with TRAIL or oxaliplatin. J Biol Chem (2010).
[115] X. Li, Q. Huang, C.N. Ong, X.F. Yang, H.M. Shen, Chrysin sensitizes tumor necrosis factor-alpha-induced apoptosis in human tumor cells via suppression of nuclear factor-kappaB. Cancer Lett 293 (2010) 109-116.
[116] A. Petronelli, G. Pannitteri, U. Testa, Triterpenoids as new promising anticancer drugs. Anticancer Drugs 20 (2009) 880-892.
[117] T.W. Day, A.R. Safa, RNA interference in cancer: targeting the anti-apoptotic protein c-FLIP for drug discovery. Mini Rev Med Chem 9 (2009) 741-748.
[118] A. Hernandez, R. Thomas, F. Smith, J. Sandberg, S. Kim, D.H. Chung, B.M. Evers, Butyrate sensitizes human colon cancer cells to TRAIL-mediated apoptosis. Surgery 130 (2001) 265-272.

[119] T.M. Ganten, R. Koschny, T.L. Haas, J. Sykora, M. Li-Weber, K. Herzer, H. Walczak, Proteasome inhibition sensitizes hepatocellular carcinoma cells, but not human hepatocytes, to TRAIL. Hepatology (Baltimore, Md 42 (2005) 588-597.
[120] M.L. Plissonnier, S. Fauconnet, H. Bittard, I. Lascombe, Insights on distinct pathways of thiazolidinediones (PPARgamma ligand)-promoted apoptosis in TRAIL-sensitive or -resistant malignant urothelial cells. Int J Cancer (2010).
[121] K. Grund, R. Ahmadi, F. Jung, V. Funke, G. Gdynia, A. Benner, J. Sykora, H. Walczak, S. Joos, J. Felsberg, G. Reifenberger, O.D. Wiestler, C. Herold-Mende, W. Roth, Troglitazone-mediated sensitization to TRAIL-induced apoptosis is regulated by proteasome-dependent degradation of FLIP and ERK1/2-dependent phosphorylation of BAD. Cancer Biol Ther 7 (2008) 1982-1990.
[122] K. Schultze, B. Bock, A. Eckert, L. Oevermann, D. Ramacher, O. Wiestler, W. Roth, Troglitazone sensitizes tumor cells to TRAIL-induced apoptosis via down-regulation of FLIP and Survivin. Apoptosis 11 (2006) 1503-1512.
[123] D. Llobet, N. Eritja, A. Yeramian, J. Pallares, A. Sorolla, M. Domingo, M. Santacana, F.J. Gonzalez-Tallada, X. Matias-Guiu, X. Dolcet, The multikinase inhibitor Sorafenib induces apoptosis and sensitises endometrial cancer cells to TRAIL by different mechanisms. Eur J Cancer 46 (2010) 836-850.
[124] S.I. Katz, L. Zhou, G. Chao, C.D. Smith, T. Ferrara, W. Wang, D.T. Dicker, W.S. El-Deiry, Sorafenib inhibits ERK1/2 and MCL-1(L) phosphorylation levels resulting in caspase-independent cell death in malignant pleural mesothelioma. Cancer Biol Ther 8 (2009) 2406-2416.
[125] T.W. Day, F. Najafi, C.H. Wu, A.R. Safa, Cellular FLICE-like inhibitory protein (c-FLIP): a novel target for Taxol-induced apoptosis. Biochem Pharmacol 71 (2006) 1551-1561.
[126] M.D. Siegelin, Y. Siegelin, A. Habel, T. Gaiser, Genistein enhances proteasomal degradation of the short isoform of FLIP in malignant glioma cells and thereby augments TRAIL-mediated apoptosis. Neurosci Lett 453 (2009) 92-97.
[127] X. Liu, P. Yue, A.H. Schonthal, F.R. Khuri, S.Y. Sun, Cellular FLICE-inhibitory protein down-regulation contributes to celecoxib-induced apoptosis in human lung cancer cells. Cancer research 66 (2006) 11115-11119.