Cancer Chemother Pharmacol (2007) 60:313–319
DOI 10.1007/s00280-006-0381-8

ORIGINAL ARTICLE

Flavopiridol synergizes TRAIL cytotoxicity by downregulation of $FLIP_L$

Tamer E. Fandy · Douglas D. Ross · Steven D. Gore · Rakesh K. Srivastava

Received: 9 March 2006 / Accepted: 29 October 2006 / Published online: 23 December 2006

Abstract
Purpose Flavopiridol is known to modulate the transcription of genes. We investigated the effect of flavopiridol pretreatment on TRAIL cytotoxicity and on the expression of $FLIP_L$ in different TRAIL-resistant cell lines, because FLIP expression is known to confer TRAIL-resistance.
Methods Apoptosis was assessed by PI staining and protein expression by Western blotting. RT-PCR was used for mRNA quantitation. siRNA gene silencing was used to knock down $FLIP_L$.
Results Flavopiridol pretreatment synergized TRAIL-induced apoptosis in human myeloma and breast cancer cells. Flavopiridol treatment repressed the transcription of $FLIP_L$ and downregulated its expression in both myeloma and breast cancer cells. Silencing of $FLIP_L$ gene by siRNA sensitized myeloma cells to TRAIL. Flavopiridol treatment downregulated the expression of the proapoptotic members of the Bcl-2 family proteins (Bak, Bax and PUMA-α). The expression of the antiapototic Bcl-2 members (Bcl-2 and Bcl-X_L) was not altered by flavopiridol treatment in myeloma cells.
Conclusion Our data indicate that flavopiridol synergizes TRAIL cytotoxicity by downregulation of $FLIP_L$ and this synergistic effect is Bcl-2 family independent.

Keywords Flavopiridol · FLIP · TRAIL · Bcl-2 family · Apoptosis

T. E. Fandy · R. K. Srivastava
School of Pharmacy,
Department of Pharmaceutical Sciences,
University of Maryland, Baltimore, MD 21201, USA

D. D. Ross · R. K. Srivastava
Greenebaum Cancer Center, School of Medicine,
University of Maryland, Baltimore, MD 21201, USA

D. D. Ross
Baltimore VA Medical Center, Baltimore, MD 21201, USA

T. E. Fandy (✉) · S. D. Gore
Sidney Kimmel Comprehensive Cancer Center,
Johns Hopkins University, 1650 Orleans St,
CRB 233, Baltimore, MD 21231, USA
e-mail: tfandy@jhu.edu

Present Address:
R. K. Srivastava
Center for Biomedical Research,
University of Texas Health Center, Tyler, TX 75708, USA

Introduction

Flavonoids, like quercetin and genistein, have been shown to arrest tumor cell growth. Flavopiridol is a new synthetic flavone that showed a potent antitumor activity both in vitro and in vivo [1, 2]. Flavopiridol acts by inhibition of cyclin-dependent kinases (cdks) [3] and was the first cdk inhibitor to enter clinical trials [4, 5]. Flavopiridol is not merely a cdk inhibitor that causes cell cycle arrest; it can induce apoptosis [1], inhibit transcription [6] and angiogenesis [7]. Flavopiridol also showed a synergistic cytotoxic effect when combined with other chemotherapeutic agents [8] and TNF-related apoptsis-inducing ligand (TRAIL) [9, 10].

The cellular FLICE-inhibitory protein (c-FLIP) is a key regulator of death receptor signaling [11]. Numerous isoforms of c-FLIP exist, of which two splice variant isoforms are mostly expressed: the short isoform

($FLIP_S$) and the long isoform ($FLIP_L$). Both isoforms have two death effector domains. The long isoform has, in addition, an inactive caspase-domain. The incorporation of $FLIP_L$ or $FLIP_S$ in the death inducing signaling complex (DISC) inhibits the autocatalytic cleavage of caspase-8/caspase-10 and consequently inhibits the activation of the effector caspases [12, 13]. Persistent or transient expression of $FLIP_L$ or $FLIP_S$ has been shown to confer resistance against TRAIL and was able to modulate TRAIL-sensitivity in prostate cancer [14] and melanoma cells [15], respectively. On the contrary, $FLIP_L$ was reported in other studies as apoptosis mediator by promoting the activation of caspase-8 and acted as apoptosis inhibitor only at high ectopic expression levels [16, 17].

In this study, we investigated the effect of flavopiridol on the expression of $FLIP_L$ and other Bcl-2 family proteins and the consequences of these effects on TRAIL cytotoxicity in TRAIL-resistant cell lines. Flavopiridol downregulated $FLIP_L$ expression in TRAIL-resistant breast cancer and multiple myeloma cell lines and synergized TRAIL-induced apoptosis. $FLIP_L$ gene silencing by siRNA sensitized TRAIL-resistant myeloma cells in a similar pattern as flavopiridol. In spite of the synergistic effect of flavopiridol on TRAIL-induced apoptosis, flavopiridol downregulated the expression of the proapoptotic members of the Bcl-2 family (PUMA, Bak and Bax) without altering the expression of the antiapoptotic members of the Bcl-2 family. Our data suggest that flavopiridol sensitizes TRAIL-resistant cells by downregulation of $FLIP_L$ in a manner not affected by the expression of the Bcl-2 family proteins.

Materials and methods

Reagents

The antibodies used were as follows: rabbit polyclonal antibodies for Bim, Bak, Bcl-X_L (Santa Cruz Biotechnology, Santa Cruz, CA, USA), PUMA-α (Imgenex, San Diego, CA, USA) and PARP (Biosource International, Camarillo, CA, USA); mouse polyclonal antibodies for Bax, Bcl-2, $FLIP_{S/L}$ (Santa Cruz Biotechnology) and β-Tubulin (Upstate, Charlottesville, VA, USA). Flavopiridol was a generous gift from Dr. Kenneth Bauer (School of Pharmacy, University of Maryland). RT-PCR primers were synthesized by Integrated DNA Technologies (Coralville, IA, USA). All other chemicals used were of analytical grade from Fisher Scientific (Suwanee, GA, USA) or Sigma (St. Louis, MO, USA).

Cell culture

MM1S myeloma cells and MDA-MB-468 breast cancer cells were cultured in RPMI 1640 medium (GIBCO, Grand Island, NY, USA) supplemented with 10% FBS (GIBCO) in a humidified incubator supplied with 5% carbon dioxide.

XTT colorimetric assay and propidium iodide (PI) staining

XTT cell proliferation assay was done as described previously [18]. Apoptosis was measured by estimating the sub-G1 population after PI staining. Cells (1×10^6) were washed twice in PBS and then fixed in 70% ethanol overnight at 4°C. Cells were washed once in PBS and then incubated with a PI solution (50 μg/ml) containing RNase at 37°C for 30 min. Sub-G1 peak was estimated using FACScan flow cytometer and CellQuest software.

Immunoblotting analysis

Immunoblotting was done as described previously [19].

RT-PCR analysis

RNA was extracted using the Qiagen RNeasy kit (Qiagen, Valencia, CA, USA). RT-PCR was performed using the Access RT-PCR system (Promega, Madison, WI, USA) as per the manufacturer's instructions. The following primers were used for c-FLIP to amplify 833 bp product; sense: 5′GCTGAAGTCA TCCATCAGGT3′, antisense: 5′CATACTGAGATG CAAGAATT3′. Bcl-2 and Bcl-X primers used were as follows, sense: 5′TTCTTTGAGTTCGGTGGGGTC3′; antisense: 5′TGCATATTTGTTTGGGGCAGG3′ and sense: 5′TTGGACAATGGACTGG TTGA3′; antisense: 5′GTAGAGTGGA TGGTCAGTG-3′, respectively. Proper loading was confirmed by using the following specific primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH): sense: 5′T CTGCCC CCTCTGCTGATGC3′; antisense: 5′CCACCACCC TGTTGCTGTAG3′.

Transfection and siRNA gene silencing

Four different c-FLIP gene-specific siRNA were designed and ligated to pSilencer hygro vector as per the manufacturer's instructions (Ambion, Austin, TX, USA). The following two complimentary oligonucleotides **ATGAAGTCAGCCCTCAGAA** and **TTCTGAGG GCTGACTTCAT** with the loop sequence **TTCAAG AGA** provided the best silencing effect as confirmed by

Western blotting. The 19 nucleotide oligo corresponds to nucleotides 329–347 of $FLIP_L$ mRNA (accession number: AF005774). Alignment of the oligonucleotide with $FLIP_S$ mRNA (accession number: AF005775) did not show any perfect similarity, indicating specificity to the long isoform only. The pSilencer vector was subcloned and used to transfect MM1S cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Scrambled siRNA was used as a negative control and was generated by the GeneBlocker™ negative control siRNA vector (Biovision Research Products, Mountain View, CA, USA).

Statisitical analyses

Results are shown as mean ± SD. Student's *t* test was used to determine significant differences. In all cases, $P < 0.05$ was considered statistically significant.

Results

Flavopiridol synergizes TRAIL-induced apoptosis and downregulates $FLIP_L$ expression in myeloma cells

We previously reported the relative resistance of MM1S myeloma cells to TRAIL cytotoxic effect in comparison to other myeloma cell lines [18]. Flavopiridol pretreatment of MM1S cells (50 and 100 nM) for 24 h followed by TRAIL (50 ng/ml) for another 24 h synergized TRAIL-induced apoptosis (Fig. 1a). The combination index (CI) values were calculated using Calcusyn software (Biosoft, UK) and were 0.217 for flavopiridol 50 nM and 0.048 for flavopiridol 100 nM, suggesting strong synergism.

The synergistic effect of flavopiridol on TRAIL was also observed in XTT survival assay (Fig. 1b) either by flavopiridol pretreatment or cotreatment for 48 h (data not shown).

The synergistic effect of flavopiridol on TRAIL-induced apoptosis was consistent with the increase in PARP cleavage after sequential treatment when compared to each drug alone (Fig. 1c).

Flavopiridol is known for its transcription inhibition effects. To investigate the effect of flavopiridol on the transcription and protein expression of both isoforms of FLIP, RT-PCR and Western blotting were used to quantitate the mRNA and protein levels of FLIP, respectively. Flavopiridol (100 nM) repressed the transcription and protein expression of $FLIP_L$ after 24 h of treatment without altering the expression of $FLIP_S$ (Fig. 1d, e, respectively).

Flavopiridol downregulates $FLIP_L$ and synergizes TRAIL-induced apoptosis in TRAIL-resistant breast cancer cells

MDA-MB-468 breast cancer cells are known for their TRAIL-resistance [19]. Sequential treatment with different doses of flavopiridol for 24 h followed by TRAIL for another 24 h synergized TRAIL-induced apoptosis (Fig. 2a). The calculated CI values for the combination were 0.2 (flavopiridol 50 nM–TRAIL 50 ng/ml) and 0.06 (flavopiridol 100 nM–TRAIL 50 ng/ml), suggesting strong synergism. The effect on $FLIP_L$ was also investigated and flavopiridol (100 nM) treatment downregulated $FLIP_L$ expression (Fig. 2b).

$FLIP_L$ gene silencing sensitizes TRAIL-resistant myeloma cells

In order to correlate $FLIP_L$ downregulation by flavopiridol to its sensitizing effect on TRAIL, $FLIP_L$ gene was silenced in MM1S myeloma cells by siRNA as described under Materials and methods and gene silencing was confirmed by Western blotting (Fig. 3a). To detect the effect of $FLIP_L$ silencing on TRAIL cytotoxicity, MM1S cells transfected with scrambled or $FLIP_L$ siRNA were treated with TRAIL (100 ng/ml) for 24 h. Downregulation of $FLIP_L$ significantly reduced MM1S cells survival in the presence of TRAIL (Fig. 3b).

Flavopiridol downregulates the proapoptotic Bcl-2 family members expression in myeloma cells

Bcl-2 family members are known for their proapoptotic and antiapoptotic effects [20, 21]. Bcl-2 and Bcl-X_L are antiapototic proteins and their overexpression significantly contributed to TRAIL-resistance [22]. On the other hand, the proapoptotic members of the Bcl-2 protein family play a major role in the disruption of the mitochondrial membrane potential $\Delta\Psi_m$ and the release of different mitochondrial proapoptotic proteins [23]. Flavopiridol (100 nM) did not alter the expression of Bcl-2 or Bcl-X_L (Fig. 4a) in spite of the transcriptional repression of the Bcl-2 gene (Fig. 4b). Moreover, flavopiridol did not modulate the transcription of Bcl-X_L in MM1S cells (Fig. 4b). Surprisingly, flavopiridol downregulated the expression of the proapoptotic Bcl-2 proteins, Bak, Bax and PUMA-α without affecting the expression of any of the three isoforms of Bim (Fig. 4a).

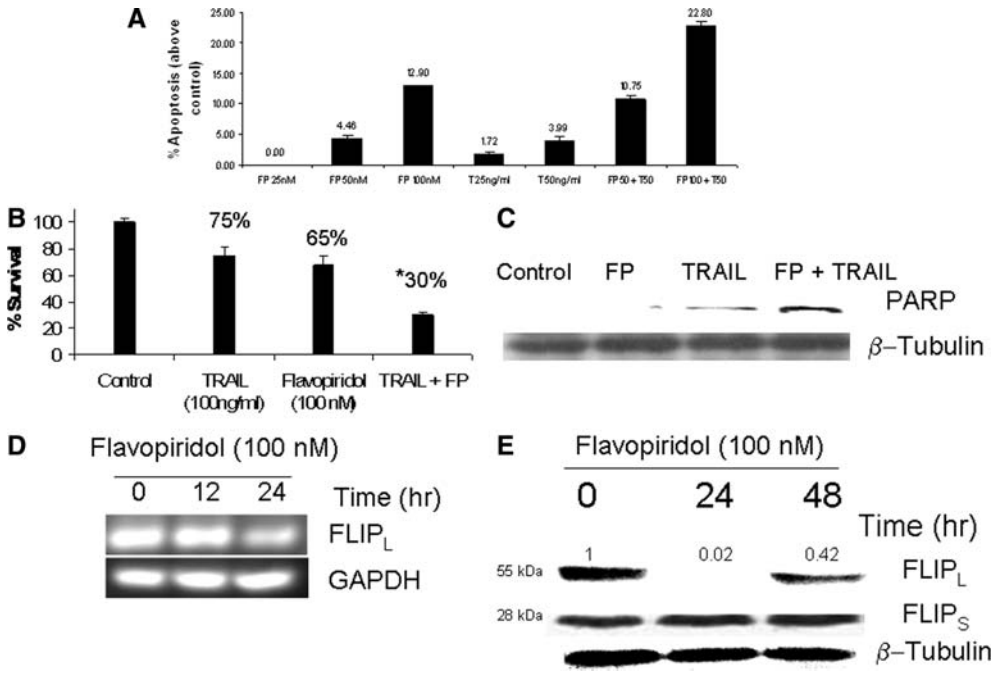

Fig. 1 Flavopiridol synergizes TRAIL cytotoxic and apoptotic effects and represses FLIP$_L$ transcription and protein expression in MM1S myeloma cells. **a** Apoptosis assay using PI staining showing dose response of flavopiridol (FP) for 48 h, TRAIL (T) for 24 h and the sequential treatment of flavopiridol (24 h) followed by TRAIL (24 h) in MM1S cells. Data represent the mean ± SD for three replicates. **b** XTT cell proliferation assay showing the enhanced effect of pretreatment of MM1S cells with flavopiridol (FP) for 24 h followed by TRAIL for another 24 h. Data represent mean ± SD for four replicates and * indicates significant difference from TRAIL or Flavopiridol treatment alone at $P < 0.05$. **c** Immunoblotting showing the effect on PARP cleavage; cells were treated with FP (100 nM) for 48 h, TRAIL (100 ng/ml) for 24 h or sequential treatment with FP for 24 h and TRAIL for 24 h. The antibody used detects only the ~85 kDa of cleaved PARP. **d** RT-PCR showing the effect of FP (100 nM) on FLIP$_L$ after different time intervals. **e** Immunoblotting showing the effect of FP (100 nM) on the expression of FLIP$_L$ and FLIP$_S$. The *values* above each band represent an arbitrary value of the signal density

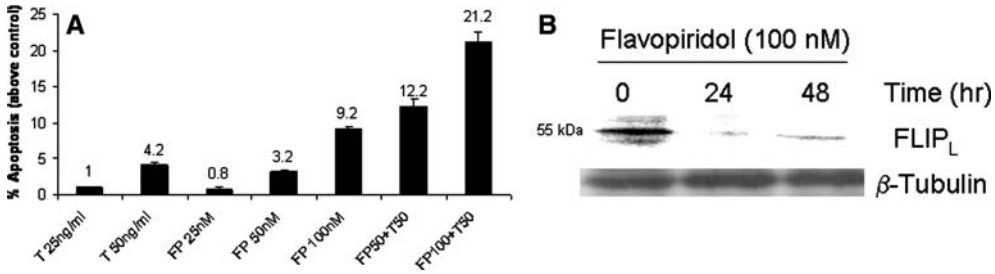

Fig. 2 Flavopiridol synergizes TRAIL-induced apoptosis and downregulates FLIP$_L$ expression in MDA-MB-468 breast cancer cells. **a** Apoptosis assessment using PI staining and sub-G1 determination in MDA-MB-468 cells using different doses of flavopiridol (FP) and TRAIL (T). Data represent mean ± SD for three replicates. **b** Immunoblotting showing the effect of flavopiridol (100 nM) on FLIP$_L$ expression in MDA-MB-468 cells

Discussion

In this study, we investigated the effect of flavopiridol on the expression of FLIP$_L$ in two TRAIL-resistant cancer cell lines and the consequences of this effect on TRAIL-resistance. Flavopiridol downregulated the expression of FLIP$_L$ in both MM1S myeloma cells and MDA-MB-468 breast cancer cells and synergized TRAIL-induced apoptosis. The sensitization of MM1S cells to TRAIL after FLIP$_L$ gene silencing strongly support the correlation between TRAIL-resistance and FLIP$_L$ expression.

The synergy between TRAIL or TNF-α and flavopiridol was reported recently [9, 10]. The synergistic effect in human leukemia cells was attributed to the downregulation of the XIAP protein (member of the inhibitor of apoptosis proteins family) by flavopiridol without affecting the expression of FLIP [10]. On the other hand, flavopiridol synergy with TNF-α was attributed to the inhibition of NF-κB transcriptional activity

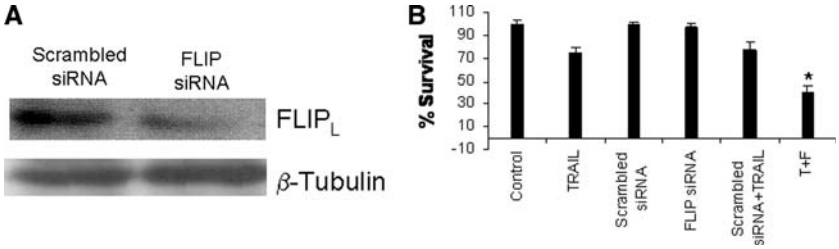

Fig. 3 Downregulation of FLIP$_L$ sensitizes MM1S TRAIL-resistant cell line. **a** Immunoblotting showing the effect of scrambled and FLIP$_L$ siRNA on the expression of FLIP$_L$. **b** XTT colorimetric assay showing the effect of TRAIL (100 ng/ml) for 24 h on FLIP$_L$ siRNA-transfected cells and scrambled-transfected cells. T + F indicates TRAIL + FLIP siRNA. Data represent mean ± SD for four replicates and * indicates significant difference from TRAIL treatment in nonscrambled-transfected cells at $P < 0.05$

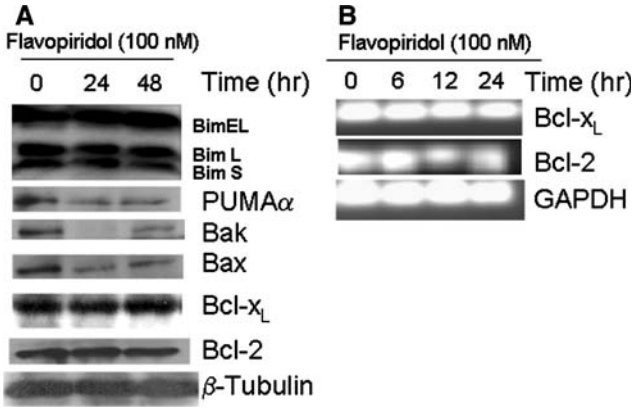

Fig. 4 Flavopiridol downregulates the expression of the proapoptotic Bcl-2 family proteins without modulating the antiapoptotic members in MM1S myeloma cells. **a** Immunoblotting showing the effect of flavopiridol (100 nM) on the expression of the proapoptotic Bcl-2 proteins (Bim; the three isoforms, Bak, Bax and PUMA α) and the antiapoptotic proteins; Bcl-2 and Bcl-X$_L$. **b** RT-PCR showing the effect of flavopiridol (100 nM) on the transcription of the antiapoptotic Bcl-2 family proteins

by flavopiridol [9]. This indicates that different intracellular factors could contribute to this synergistic effect. We also demonstrated in this study the synergistic effect of flavopiridol on TRAIL. However, we observed the downregulation of FLIP$_L$ and established a correlation between FLIP$_L$ and TRAIL-resistance by silencing the FLIP$_L$ gene. In support of this correlation, persistent FLIP$_L$ overexpression was shown to confer TRAIL-resistance in prostate cancer [14].

The observed downregulation of FLIP$_L$ by flavopiridol in myeloma cells was consistent with the observed decrease in its mRNA level. However, the lack of a quantitative correlation between mRNA transcript level and protein expression does not support this as a proposed mechanism for FLIP$_L$ downregulation. Other possible mechanisms like increased protein degradation via the ubiquitin-proteasome pathway needs further investigation.

The effect of flavopiridol on the expression of the Bcl-2 family has been controversial. Downregulation and transcriptional repression of Bcl-2 by flavopiridol was reported previously [24]. Another group reported the same effect at high concentrations of flavopiridol (>100 nM) without any change at lower concentrations [25]. However, these findings of Bcl-2 downregulation were not confirmed by others [26–28]. In our study, we could not detect any downregulation of Bcl-2 or Bcl-X$_L$, which is in agreement with the Bcl-2 independence of flavopiridol-induced apoptosis [28]. The downregulation of Bak, Bax and PUMA-α does not contradict with the observed TRAIL-enhancing effect, because TRAIL-induced apoptosis can be executed in a mitochondrial-independent manner through the extrinsic pathway.

The possibility that the observed synergistic effect of flavopiridol on TRAIL is due to multiple factors and not solely attributed to the downregulation of FLIP$_L$ cannot be ruled out. Other proteins like XIAP, survivin, c-IAP1 could be downregulated and contribute to this effect. However, the observation that FLIP$_L$ downregulation by siRNA doubled the cytotoxic effect of TRAIL indicates that FLIP$_L$ plays a major role in TRAIL-resistance in these cells. Moreover, during the revision process of this manuscript, another study [29] reported the same effect of flavopiridol in several breast cancer cells and found that XIAP gene silencing or abrogation of NF-κB activity were not enough to sensitize breast cancer cells to TRAIL, which support a dominant role of FLIP expression over XIAP expression and NF-κB activity in the observed flavopiridol synergy.

Mcl-1 is a member of the antiapoptotic Bcl-2 family and thought to play a key role in mediating drug resistance in multiple myeloma. Flavopiridol was shown to repress the transcription and downregulate Mcl-1 expression in other myeloma cell lines [30]. Cholangiocarcinoma cells that overexpress Mcl-1 and exhibit TRAIL resistance were sensitized to TRAIL by Mcl-1

gene silencing [31], further demonstrating the role of Mcl-1 in mediating TRAIL resistance. We did not observe any change in Mcl-1 expression in MM1S cells after 24 and 48 h treatment with flavopiridol (data not shown). The reported effect of flavopiridol on Mcl-1 was detected at early time points (6 h) and then Mcl-1 expression was almost restored to the basal level at 14 h [30], which may explain the observed unaltered expression of Mcl-1 after 24 h in our study. Furthermore, the higher dose of flavopiridol (200 nM vs. 100 nM in this study) and the different cell line used may contribute to this difference.

In conclusion, this study showed that (1) flavopiridol repressed the transcription and protein expression of $FLIP_L$, (2) flavopiridol synergized TRAIL-induced apoptosis in a Bcl-2 independent manner and (3) $FLIP_L$ downregulation could be a useful strategy in sensitizing TRAIL-resistant cells.

References

1. Bible KC, Kaufmann SH (1996) Flavopiridol: a cytotoxic flavone that induces cell death in noncycling A549 human lung carcinoma cells. Cancer Res 56:4856–4861
2. Drees M, Dengler WA, Roth T, Labonte H, Mayo J, Malspeis L, Grever M, Sausville EA, Fiebig HH (1997) Flavopiridol (L86–8275): selective antitumor activity in vitro and activity in vivo for prostate carcinoma cells. Clin Cancer Res 3:273–279
3. Carlson BA, Dubay MM, Sausville EA, Brizuela L, Worland PJ (1996) Flavopiridol induces G1 arrest with inhibition of cyclin-dependent kinase (CDK) 2 and CDK4 in human breast carcinoma cells. Cancer Res 56:2973–2978
4. Shapiro GI, Supko JG, Patterson A, Lynch C, Lucca J, Zacarola PF, Muzikansky A, Wright JJ, Lynch TJ Jr, Rollins BJ (2001) A phase II trial of the cyclin-dependent kinase inhibitor flavopiridol in patients with previously untreated stage IV non-small cell lung cancer. Clin Cancer Res 7:1590–1599
5. Schwartz GK, Ilson D, Saltz L, O'Reilly E, Tong W, Maslak P, Werner J, Perkins P, Stoltz M, Kelsen D (2001) Phase II study of the cyclin-dependent kinase inhibitor flavopiridol administered to patients with advanced gastric carcinoma. J Clin Oncol 19:1985–1992
6. Chao SH, Fujinaga K, Marion JE, Taube R, Sausville EA, Senderowicz AM, Peterlin BM, Price DH (2000) Flavopiridol inhibits P-TEFb and blocks HIV-1 replication. J Biol Chem 275:28345–28348
7. Melillo G, Sausville EA, Cloud K, Lahusen T, Varesio L, Senderowicz AM (1999) Flavopiridol, a protein kinase inhibitor, down-regulates hypoxic induction of vascular endothelial growth factor expression in human monocytes. Cancer Res 59:5433–5437
8. Schwartz GK, Farsi K, Maslak P, Kelsen DP, Spriggs D (1997) Potentiation of apoptosis by flavopiridol in mitomycin-C-treated gastric and breast cancer cells. Clin Cancer Res 3:1467–1472
9. Kim DM, Koo SY, Jeon K, Kim MH, Lee J, Hong CY, Jeong S (2003) Rapid induction of apoptosis by combination of flavopiridol and tumor necrosis factor (TNF)-alpha or TNF-related apoptosis-inducing ligand in human cancer cell lines. Cancer Res 63:621–626
10. Rosato RR, Dai Y, Almenara JA, Maggio SC, Grant S (2004) Potent antileukemic interactions between flavopiridol and TRAIL/Apo2L involve flavopiridol-mediated XIAP downregulation. Leukemia 18:1780–1788
11. Irmler M, Thome M, Hahne M, Schneider P, Hofmann K, Steiner V, Bodmer JL, Schroter M, Burns K, Mattmann C et al (1997) Inhibition of death receptor signals by cellular FLIP. Nature 388:190–195
12. Shain KH, Landowski TH, Dalton WS (2002) Adhesion-mediated intracellular redistribution of c-Fas-associated death domain-like IL-1-converting enzyme-like inhibitory protein-long confers resistance to CD95-induced apoptosis in hematopoietic cancer cell lines. J Immunol 168:2544–2553
13. Srivastava RK (2001) TRAIL/Apo-2L. Mechanisms and clinical applications in cancer. Neoplasia 3:535–546
14. Zhang X, Jin TG, Yang H, DeWolf WC, Khosravi-Far R, Olumi AF (2004) 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 Res 64:7086–7091
15. Zeise E, Weichenthal M, Schwarz T, Kulms D (2004) Resistance of human melanoma cells against the death ligand TRAIL is reversed by ultraviolet-B radiation via downregulation of FLIP. J Invest Dermatol 123:746–754
16. Chang DW, Xing Z, Pan Y, Algeciras-Schimnich A, Barnhart BC, Yaish-Ohad S, Peter ME, Yang X (2002) c-FLIP(L) is a dual function regulator for caspase-8 activation and CD95-mediated apoptosis. EMBO J 21:3704–3714
17. Micheau O, Thome M, Schneider P, Holler N, Tschopp J, Nicholson DW, Briand C, Grutter MG (2002) The long form of FLIP is an activator of caspase-8 at the Fas death-inducing signaling complex. J Biol Chem 277:45162–45171
18. Fandy TE, Shankar S, Ross DD, Sausville E, Srivastava RK (2005) Interactive effects of HDAC inhibitors and TRAIL on apoptosis are associated with changes in mitochondrial functions and expressions of cell cycle regulatory genes in multiple myeloma. Neoplasia 7:646–657
19. Singh TR, Shankar S, Chen X, Asim M, Srivastava RK (2003) Synergistic interactions of chemotherapeutic drugs and tumor necrosis factor-related apoptosis-inducing ligand/Apo-2 ligand on apoptosis and on regression of breast carcinoma in vivo. Cancer Res 63:5390–5400
20. Srivastava RK, Mi QS, Hardwick JM, Longo DL (1999) Deletion of the loop region of Bcl-2 completely blocks paclitaxel-induced apoptosis. Proc Natl Acad Sci USA 96:3775–3780
21. Srivastava RK, Sollott SJ, Khan L, Hansford R, Lakatta EG, Longo DL (1999) Bcl-2 and Bcl-X(L) block thapsigargin-induced nitric oxide generation, c-Jun NH(2)-terminal kinase activity, and apoptosis. Mol Cell Biol 19:5659–5674
22. Burns TF, El-Deiry WS (2001) Identification of inhibitors of TRAIL-induced death (ITIDs) in the TRAIL-sensitive colon carcinoma cell line SW480 using a genetic approach. J Biol Chem 276:37879–37886
23. Narita M, Shimizu S, Ito T, Chittenden T, Lutz RJ, Matsuda H, Tsujimoto Y (1998) Bax interacts with the permeability transition pore to induce permeability transition and cytochrome c release in isolated mitochondria. Proc Natl Acad Sci USA 95:14681–14686
24. Konig A, Schwartz GK, Mohammad RM, Al-Katib A, Gabrilove JL (1997) The novel cyclin-dependent kinase inhibitor flavopiridol downregulates Bcl-2 and induces growth arrest and apoptosis in chronic B-cell leukemia lines. Blood 90:4307–4312

25. Robinson WA, Miller TL, Harrold EA, Bemis LT, Brady BM, Nelson RP (2003) The effect of flavopiridol on the growth of p16+ and p16− melanoma cell lines. Melanoma Res 13:231–238
26. Schrump DS, Matthews W, Chen GA, Mixon A, Altorki NK (1998) Flavopiridol mediates cell cycle arrest and apoptosis in esophageal cancer cells. Clin Cancer Res 4:2885–2890
27. Byrd JC, Shinn C, Waselenko JK, Fuchs EJ, Lehman TA, Nguyen PL, Flinn IW, Diehl LF, Sausville E, Grever MR (1998) Flavopiridol induces apoptosis in chronic lymphocytic leukemia cells via activation of caspase-3 without evidence of bcl-2 modulation or dependence on functional p53. Blood 92:3804–3816
28. Achenbach TV, Muller R, Slater EP (2000) Bcl-2 independence of flavopiridol-induced apoptosis. Mitochondrial depolarization in the absence of cytochrome c release. J Biol Chem 275:32089–32097
29. Palacios C, Yerbes R, Lopez-Rivas A (2006) Flavopiridol induces cellular FLICE-inhibitory protein degradation by the proteasome and promotes TRAIL-induced early signaling and apoptosis in breast tumor cells. Cancer Res 66:8858–8869
30. Gojo I, Zhang B, Fenton RG (2002) The cyclin-dependent kinase inhibitor flavopiridol induces apoptosis in multiple myeloma cells through transcriptional repression and down-regulation of Mcl-1. Clin Cancer Res 8:3527–3538
31. Taniai M, Grambihler A, Higuchi H, Werneburg N, Bronk SF, Farrugia DJ, Kaufmann SH, Gores GJ (2004) Mcl-1 mediates tumor necrosis factor-related apoptosis-inducing ligand resistance in human cholangiocarcinoma cells. Cancer Res 64:3517–3524