Biochemical and Biophysical Research Communications 428 (2012) 185–190

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Carnitine sensitizes TRAIL-resistant cancer cells to TRAIL-induced apoptotic cell death through the up-regulation of Bax

So Jung Park [a,1], Seong Ho Park [b,1], Joo-Oh Kim [a], Jung Ho Kim [a], So Jung Park [c], Jung Jin Hwang [c,d], Dong-Hoon Jin [c,d], Seong-Yun Jeong [c,d], Seung Jin Lee [c,d], Jin Cheon Kim [d,e], InKi Kim [c,*], Dong-Hyung Cho [a,*]

[a] Graduate School of East-West Medical Science, Kyung Hee University, Gyeoggi-do, South Korea
[b] Ilsong Institute for Life Science, Hallym University, Kangwon-do, South Korea
[c] Asan Institute for Medical Research, University of Ulsan College of Medicine, Asan Medical Center, Seoul, South Korea
[d] Institute for Innovative Cancer Research, Asan Medical Center, Seoul, South Korea
[e] Department of Surgery, University of Ulsan College of Medicine, Asan Medical Center, Seoul, South Korea

ARTICLE INFO

Article history:
Received 24 September 2012
Available online 13 October 2012

Keywords:
TRAIL
Resistance
Carnitine
A549
Apoptosis
Sensitization

ABSTRACT

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a member of the tumor necrosis factor family with apoptosis-inducing activity. Given that TRAIL selectively induces cell death in various tumors but has little or no toxicity to normal cells, TRAIL agonists have been considered as promising anti-cancer therapeutic agents. However, the resistance of many primary tumors and cancer cells to TRAIL poses a challenge. In our present study, we found that carnitine, a metabolite that transfers long-chain fatty acids into mitochondria for beta-oxidation and modulates protein kinase C activity, sensitizes TRAIL-resistant cancer cells to TRAIL. Combination of carnitine and TRAIL was found to synergistically induce apoptotic cell death through caspase activation, which was blocked by a pan caspase inhibitor, but not by an inhibitor of autophagy or an inhibitor of necrosis. The combination of carnitine and TRAIL reversed the resistance to TRAIL in lung cancer cells, colon carcinoma cells, and breast carcinoma cells. We further demonstrate that carnitine, either alone or in combination with TRAIL, enhances the expression of the pro-apoptotic Bcl-2 family protein, Bcl-2-associated X protein (Bax). The down-regulation of Bax expression by small interfering RNA reduced caspase activation when cells were treated with TRAIL, and experiments with cells from Bax knockout mice confirmed this result. Taken together, our current results suggest that carnitine can reverse the resistance of cancer cells to TRAIL by up-regulating Bax expression. Thus, a combined delivery of carnitine and TRAIL may represent a new therapeutic strategy to treat TRAIL-resistant cancer cells.

1. Introduction

The tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a member of the tumor necrosis factor family of cytokines. The binding of TRAIL to its receptors, death receptor 4 (DR4) and DR5, triggers apoptotic signaling. The activation of DR4 or DR5 recruits the Fas-associated death domain protein (FADD) and procaspase-8 to form the death-inducing signaling complex (DISC), which leads to the activation of the caspase cascade [1,2]. Caspase activation can be suppressed by the inhibitor of apoptosis protein (IAP) family members, as well as by anti-apoptotic B-cell lymphoma 2 (Bcl-2) family proteins [3].

The ability of TRAIL to selectively induce cell death in various tumors, whilst showing little or no toxicity to normal cells, has spurred several clinical development initiatives to test TRAIL for cancer therapy [4]. However, most primary tumors and a range of patient-derived cancer cell lines eventually become resistant to TRAIL. For example, approximately 50% of all non-small cell lung cancer (NSCLC) cells are resistant to the apoptotic effects of TRAIL [5]. Resistance to TRAIL can arise from either dysfunctional TRAIL receptors, defects in DISC proteins, or the abnormal expression of either anti-apoptotic or pro-apoptotic proteins [6,7]. Hence, an understanding of the molecular mechanisms of TRAIL resistance, and thereby overcoming it, is a major challenge for the development of effective TRAIL-based therapeutic strategies. Numerous studies have indicated that a combined delivery of TRAIL and chemotherapeutic agents sensitizes cells to TRAIL through

* Corresponding authors. Addresses: Graduate School of East-West Medical Science, Kyung Hee University, 1, Seocheon-dong, Giheung-gu, Yongin-si, Gyeoggi-do 446-701, South Korea (D.-H. Cho). Asan Institute for Medical Reserch, Asan Medical Center, Seoul 138-736, South Korea (I. Kim).
E-mail addresses: ik.kim@amc.seoul.kr (I. Kim), dhcho@khu.ac.kr (D.-H. Cho).
[1] These authors contributed equally.

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.10.038

mechanisms such as the up-regulation of death receptors, and regulation of Bcl-2 protein expression [8,9].

Our previous efforts to find a novel TRAIL sensitizer involved simple screening of a bio-active chemical library, using TRAIL-resistant cells [10]. From this endeavor, we identified L-carnitine (carnitine) as a strong TRAIL sensitizer. Carnitine is an essential cofactor in fatty acid metabolism, and regulates the activity of protein kinase C (PKC) [11–13]. In this study, we investigated the effects of carnitine on cell death in TRAIL-resistant cancer cells and found that it strongly sensitizes these cells to TRAIL-induced apoptosis through the up-regulation of the Bax protein.

2. Materials and methods

2.1. Cells

Cancer cells originating from lung (A549, H460, and H129), colon (HCT116, HT29, and SW620) and breast (MCF-7) tumors were purchased from the American Type Culture Collection (ATCC). These cells were maintained in Roswell Park Memorial Institute (RPMI) or McCoy's medium supplemented with 10% FBS and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA). HCT116 Bax null cells were kindly provided by Dr. Bert Vogelstein (Johns Hopkins University, MD).

2.2. Reagents

Recombinant human TRAIL and zVAD-FMK were purchased from R&D Systems (Minneapolis, MN). L-Carnitine, bafilomycin, and necrostatin-1 were obtained from Sigma–Aldrich (St. Louis, MO). A siRNA previously shown to target Bax (5′-AACATGGAGCTGCAGAGGAT-3′) [14] and a negative scrambled siRNA (5′-CCUACGCCACCAAUUUCGU-3′) were synthesized by Bioneer corporation (Daejeon, Korea).

2.3. Cell viability assay

Cell viability was determined using a cell counting kit-8 (CCK-8) in accordance with the manufacturer's protocol (Dojindo Corporation, Japan). Briefly, cells incubated with a test compound in a 96-well plate received 10 μl of CCK-8 solution, and were then incubated for 1 h in a CO_2 incubator. The subsequent colorimetric change was measured using a Victor microtiter plate reader (PerkinElmer) set to monitor changes in absorbance at 450 nm.

2.4. Western blotting

All cells lysates were prepared with 2 × Laemmli sample buffer (62.5 mM Tris–HCl, pH 6.8, 25% glycerol, 2% SDS, 5% β-mercaptoethanol, 0.01% bromophenol blue) (BioRad, Hercules, CA). Proteins (approximately 50 ug) were quantitated by using the Bradford solution (BioRad) according to the manufacture's instruction. Then the samples were separated by SDS–polyacrylamide gel electrophoresis, and transferred to PVDF membrane (BioRad). After blocking with 4% skim milk in TBST (25 mM Tris, 3 mM 140 mM NaCl, 0.05% Tween 20), the membranes were incubated over-night with specific primary antibodies; anti-actin (MAB1501) antibody was obtained from Millipore (Temecula, USA); Anti-Bcl-xL (#2764), anti-Bax (#2772), anti-Bid (#2002), anti-Bik (#4592), anti-cleaved caspase-3 (#9661), anti-Mcl-1(#5453) antibodies were all purchased from Cell Signaling Technology (Danvers, MA); anti-DR4 (ab13890) and anti-DR5 (ab47179) antibodies were sourced from Abcam (Cambridge, MA); an antibody against caspase-10 (IMG-4150) was obtained from Imgenex (San Deigo, CA); anti-XIAP (61062) antibody was obtained from BD Bioscience (San Jose, CA); and the antibody against caspase-8 used in our experiment was generated in our laboratory. For protein detection, the membranes were incubated with HRP-conjugated secondary antibodies (Pierce, Rockford, IL).

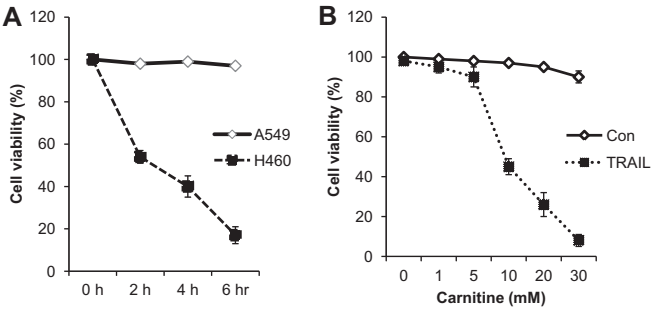

Fig. 1. Carnitine potentiates TRAIL-induced cell death in A549 cells. (A) Cytotoxic activity of TRAIL in lung cancer cell lines. Both A549 and H460 cells were treated with TRAIL (20 ng/ml) for the times indicated, and cell viability was measured by CCK-8 assay. (B) Carnitine sensitizes A549 cells to TRAIL-induced cell death. The A549 cells were incubated with different concentrations of carnitine (1–30 mM) in the presence (closed box) or absence (Con; open diamond) of TRAIL (20 ng/m) for 6 h. Cell viability was then determined by CCK-8 assay. The data are presented as the means ± standard error (SEM) ($n = 3$).

2.5. Statistical analysis

Values are presented as the means ± standard error of the mean (SEM) from at least three independent experiments. Statistical evaluation of the results was performed using one-way ANOVA.

3. Results

3.1. Carnitine potentiates TRAIL-induced cell death in TRAIL-resistant A549 cells

Although TRAIL is a promising anti-cancer agent that preferentially kills tumor cells, most primary tumors and cancer cells derived from cancer patients are resistant to TRAIL-induced cell death. For instance, the A549 lung adenocarcinoma cell line is resistant to the apoptotic effects of TRAIL [8]. Consistent with this earlier report, treatment with TRAIL induced limited cell death in A549 cells compared with that of H460 lung adenocarcinoma cells. This indicates that A549 cells are highly resistant to TRAIL-induced cell death (Fig. 1A). To identify new sensitizers of TRAIL, we previously screened a library that contains 2480 bioactive small molecules (LOPAC1280™ from Sigma, and the Prestwick 1200 collection from Prestwick) and identified carnitine as a TRAIL sensitizer [10].

To confirm our earlier screening results, A549 cells were treated with differential doses of carnitine with or without TRAIL (Fig. 1B). Co-treatment of carnitine with TRAIL significantly increased cell death in these cultures, suggesting that carnitine may sensitize A549 cells to TRAIL. We next examined the activation of caspases that mainly mediate TRAIL-induced cell death. Whereas treatment of A549 cells with either TRAIL alone or carnitine alone minimally activates caspase-8 or caspase-3, combined treatment with both TRAIL with carnitine strongly activated both caspase-3 and caspase-8 (Fig. 2A).

Given that cell death can be classified as one of three different types—apoptotic, necrotic, and autophagic—we next investigated the cell death mechanism induced by three specific inhibitors: zVAD, a pan caspase inhibitor; bafilomycin, an inhibitor of the maturation of autophagosomes to autolysosomes; and necrostatin-1, which inhibits necrotic cell death. The A549 cells were exposed

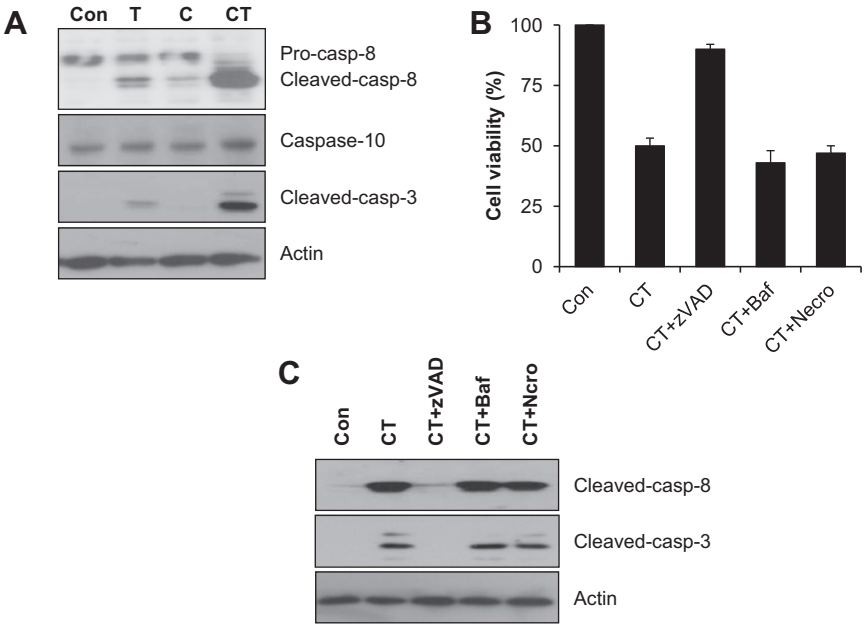

Fig. 2. A combination of carnitine and TRAIL induces apoptotic cell death in A549 cells. (A) Activation of caspases by a combined delivery of carnitine and TRAIL. The A549 cells were treated for six hours with TRAIL (*T*, 20 ng/ml), carnitine (10 mM), or a combination of the same concentrations of carnitine and TRAIL (CT). The cells were then harvested, and analyzed by Western blotting with antibodies against the proteins indicated. Actin was used as an internal loading control. (B) and (C) Suppression of CT-induced cell death by zVAD. A549 cells were exposed to CT with or without the cell death inhibitors zVAD (40 μM), bafilomycin (Baf, 100 nM), or necrostatin-1 (Necro, 10 μM). Cell viability and caspase activation were then analyzed by CCK-8 assay (B) and western blotting (C). The data are presented as the means ± SEM ($n = 3$).

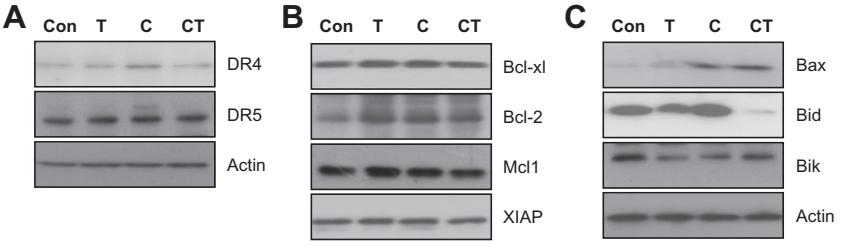

Fig. 3. Carnitine induces Bax expression in A549 cells. After A549 cells were treated for 6 h with TRAIL (T, 20 ng/ml), carnitine (10 mM), or a combination of carnitine and TRAIL at the same concentrations (CT), the cells were then harvested for western blotting using antibodies against the DR4 and DR5 death receptors (A), the anti-apoptotic Bcl-2 proteins Bcl-xl, Bcl-2, Mcl1, and XIAP (B), and the pro-apoptotic Bcl-2 proteins Bax, Bid, and Bik (C).

to both carnitine and TRAIL with or without each of these three inhibitors. Inhibition of caspase by zVAD treatment notably suppressed the cell death induced by the combination of carnitine and TRAIL, whereas bafilomycin or necrostatin-1 did not have this effect (Fig. 2B). Consistently, caspase-8 and caspase-3 activation were only inhibited in zVAD-treated cells (Fig. 2C), implying that the combination of carnitine and TRAIL induces caspase-dependent apoptotic cell death.

3.2. Carnitine increases Bax expression in A549 cells

Resistance to TRAIL can be conferred at multiple levels, including at the sites of receptors upstream and downstream of the caspase cascade. To further investigate the mechanism responsible for carnitine-mediated sensitization to TRAIL-induced apoptosis, we examined the expression of TRAIL receptors and anti-apoptotic and pro-apoptotic Bcl-2 family proteins (Fig. 3A–C). The expression levels of TRAIL receptors such as DR4 and DR5 were not altered by treatment with carnitine alone or by a combination of carnitine and TRAIL. Similarly, the expression of anti-apoptotic proteins, including Bcl-2, Bcl-X_L, Mcl-1, and XIAP protein, was also unaltered. However, Bax expression was highly increased in carnitine-treated cells (Fig. 3C). Bid is a well known substrate for caspase-8 during apoptosis [15]. Thus, down-regulation of bid protein also indicated that the combination of carnitine and TRAIL promotes apoptotic cell death. Interestingly, it was recently reported that combination treatment of carnitine and butyrate increases the expression of Bax in colon cancer cells [16]. Consistent with the findings of this earlier report, our current results suggest that Bax is associated with the ability of carnitine to sensitize cells to TRAIL.

3.3. The ablation of Bax expression decreases TRAIL- induced caspase activation

Given that carnitine treatment was found to increase Bax expression, we next investigated the effects of the combination of carnitine and TRAIL in cells in which Bax expression was silenced. This combined treatment induced caspase-3 activation and Bax accumulation in cells transfected with scrambled siRNA. However, the level of caspase-3 activation was lower in cells transfected with Bax siRNA than in control cells (Fig. 4A). We further investigated caspase activation in Bax knockout cells treated with a combination of carnitine and TRAIL. This treatment of HCT116 cells and Bax-null HCT116 cells caused activation of caspase-3 (Fig. 4B). The lower rate of caspase-3 cleavage in the Bax-null cells

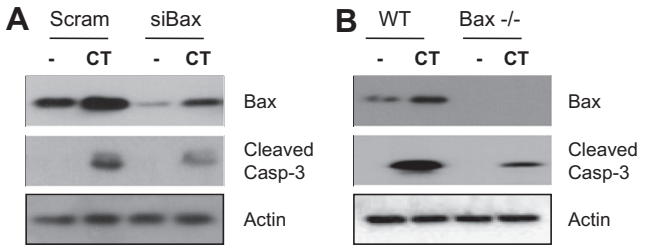

Fig. 4. Silencing of Bax expression reduces caspase activation in cells treated with a combination of carnitine and TRAIL. (A) 3 days after A549 cells were transiently transfected with scrambled negative siRNA (Scram) or Bax specific siRNA (siBax), the cells were treated with a combination of carnitine (10 mM) and TRAIL (20 ng/ml) for 6 h. The cells were then harvested, and subjected to western blot analysis with antibodies against Bax, active caspase-3, and actin. (B) Wild type (WT) and Bax knockout ($Bax^{-/-}$) HCT116 cells were treated with a combination of carnitine (10 mM) and TRAIL (20 ng/ml) for 4 h. The cells were then harvested, and subjected to western blot analysis with antibodies against Bax and active caspase-3. Actin was used as internal loading control.

compared with the control HCT116 cells in this experiment suggests that Bax is a critical mediator of the cell death triggered by the combination of carnitine and TRAIL.

3.4. Carnitine reverses the TRAIL resistance of various cancer cells

We next investigated whether the sensitizing effect of carnitine with TRAIL is specific to A549 cells, or is also effective in other TRAIL-resistant cancer cells. We treated H1299 lung adenocarcinoma cells, MCF-7 breast adenocarcinoma cells, SW620 colorectal adenocarcinoma cells, and HT29 colorectal adenocarcinoma cells either with carnitine alone, TRAIL alone, or a combination of carnitine and TRAIL. Consistent with the results obtained using A549 cells, treatment with a combination of carnitine and TRAIL synergistically induced cell death in all of these additional cancer cell types (Fig. 5A–D). These results suggest that carnitine sensitizes a range of TRAIL-resistant cancer cells to TRAIL-induced cell death.

4. Discussion

In our current study, we demonstrate that carnitine potentiates TRAIL-induced apoptosis in TRAIL-resistant cancer cells. Carnitine plays roles in energy production as a cofactor in fatty acid metabolism and regulates the transport of long chain fatty acids across the inner mitochondrial membrane prior to their catabolism through β-oxidation. Moreover, carnitine and its analogs are involved in cell death. As an antioxidant, carnitine suppresses neurotoxicity and cardiovascular risk [11,17,18]. Carnitine also suppresses apoptosis in skeletal muscle cells and prevents skeletal muscle myopathy following heart failure [17]. However, carnitine can selectively kill cancer cells while not harming normal cells. For example, carnitine induces apoptosis in HT29, U936, and Hepa1c1c7 cancer cells but not in a normal NCTC1469 cell line [19–21]. The ability of carnitine to inhibit hepatocarcinogenesis in rat models also suggests that carnitine has different functions in different cell types [12]. Kong et al. showed that a low concentration of carnitine (less than 5 mM) did not affect cell viability, whereas a high concentration (30 mM) of carnitine significantly induced apoptosis of myocytes [22]. Similarly, we also observed in our present analyses that carnitine alone, at a concentration of 30 mM but not at 10 mM, slightly reduces the viability of A549 cells. On the other hand, when combined with TRAIL, 10 mM carnitine strongly induced cell death in A549 cells (Fig. 1).

The feature of TRAIL that makes it such a promising anticancer agent is its ability to preferentially trigger apoptosis in tumor cells. Accordingly, the therapeutic potential of TRAIL is currently being tested in various clinical trials [23]. However, the fact that most primary tumors are resistant to TRAIL highlights the importance of elucidating the mechanism(s) that underlie this resistance and identifying new sensitizers that will enhance the therapeutic potential of TRAIL [23]. Several molecular mechanisms of TRAIL-resistance have now been proposed. Functional defects in TRAIL receptors, including DR4 and DR5, are one possibility [24]. Thus, an increased abundance of the receptors would likely potentiate

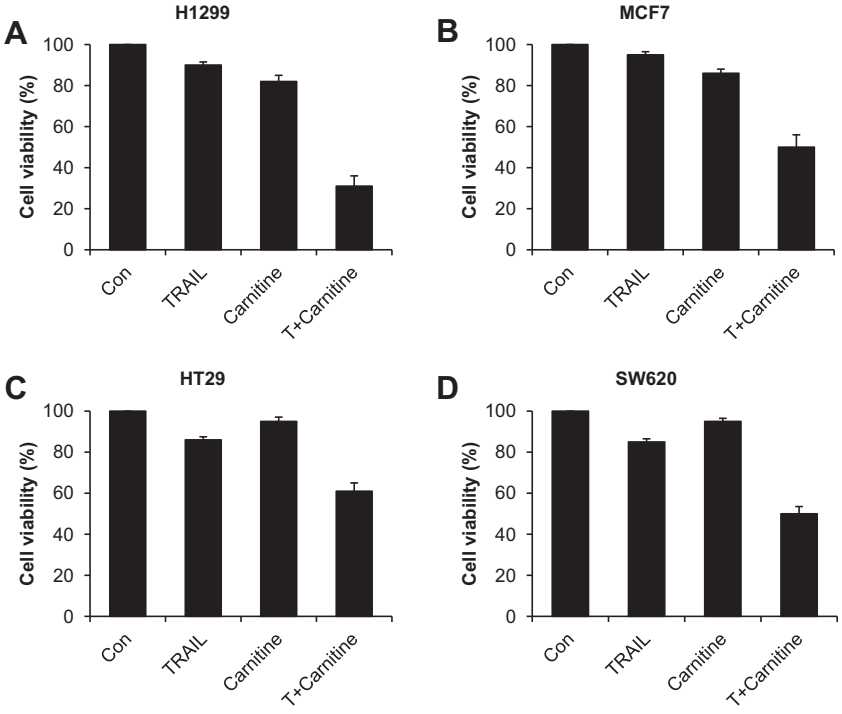

Fig. 5. Carnitine sensitizes TRAIL-resistant cancer cells to TRAIL-induced cell death.The H1299 (A), MCF7 (B), HT29 (C), and SW620 (D) cancer cell lines were treated with TRAIL (20 ng/ml) alone, carnitine (10 mM) alone, or a combination of TRAIL and carnitine (*T* + Carnitine) for 6 h Control treatments (Con) involved neither carnitine nor TRAIL. Cell viability was determined by CCK-8 assay. The data are presented as the means ± SEM ($n = 3$).

the susceptibility of cancer cells to TRAIL-induced apoptosis [8]. To investigate this possibility, we studied the effects of carnitine on the expression of both DR4 and DR5 receptors in A549 cells, but found no effect either alone or in combination with TRAIL (Fig. 3). Another possible explanation of TRAIL resistance is that it is associated with particular characteristics of tumor cells, such as the ability to avoid cell death [25]. Tumors suppress apoptosis in a general manner through mechanisms such as enhanced expression of anti-apoptotic proteins and reduced expression of pro-apoptotic proteins. For example, anti-apoptotic proteins such as Bcl-2 family proteins, IAP family proteins, and FLICE-inhibitory proteins (FLIP proteins) are highly up-regulated in several tumor cells [26–30]. Various chemotherapeutic agents like HDAC inhibitors and proteasome inhibitors are often reducing expressional level of these anti-apoptotic molecules [31,32]. Therefore, it has been suggested that a combination of these agents with TRAIL could be an effective strategy for targeting and killing TRAIL-resistant cancer cells. To investigate the mechanism by which carnitine reverses TRAIL-resistance, we examined the expression levels of several pro-apoptotic and anti-apoptotic proteins. Interestingly, we found that Bax was notably increased by treatment with carnitine, either alone or in combination with TRAIL, whilst the expression of other anti-apoptotic proteins was not significantly changed.

Apoptosis is controlled by the functional interaction of Bcl-2 family proteins, which include anti-apoptotic Bcl-2 like members (e.g., Bcl-2, Bcl-xL, Bcl-w, Mcl-1, Boo, and A1 protein), pro-apoptotic Bax-like proteins (e.g., Bax, Bak, Bcl-xS, and Bok), and pro-apoptotic BH3-only proteins (e.g., Bad, Bid, Bim, Bik, Blk, Hrk, Noxa, and Puma) [33]. The relative abundance of these pro-apoptotic and anti-apoptotic Bcl-2 proteins is critical to the appropriate regulation of apoptotic responses in the cell. The interaction of BH3-only proteins with anti-apoptotic Bcl-2 proteins effectively neutralizes their survival-promoting activities. Moreover, the oligomerization of Bax, induced by BH3-only proteins, and the subsequent permeabilization of the outer mitochondrial membrane initiates the caspase cascade. In addition, Bax expression could be increased by transcriptional regulation during cell death [34]. Although we could not demonstrate the precise mechanism by which carnitine increases the expression of Bax, the findings of recent reports have indicated that pretreatment with the carnitine derivative acetyl-L-carnitine abolishes the decrease in p53 protein abundance triggered by 3-nitropropionic acid [35]. In addition, carnitine also increases the expression of the p53 and CD95 genes in doxorubicin-induced cardiomyopathy in rats [36]. The p53 tumor suppressor regulates apoptosis in a number of different cellular contexts. The Bax protein is a target of p53 transcription factor activity and Bax induction has been observed during p53-mediated apoptosis [37,38]. Furthermore, it has been reported that carnitine also increases the expression of caspase-8 and caspase-9, as well as TNF-α, at the mRNA level [21]. Thus, the mechanisms that underlie the effects of both p53 and carnitine during the up-regulation of Bax remain to be further elucidated.

In conclusion, we here report that carnitine induces synergistic apoptosis in A549 cells via the increased expression of the pro-apoptotic Bax protein. In addition, the combination of carnitine and TRAIL strongly potentiates apoptosis in various TRAIL-resistant cell lines. These insights suggest that simultaneous administration of carnitine and TRAIL may be an efficient approach to overcoming TRAIL resistance in many tumor types.

Acknowledgments

We thank to Dr. Bert Vogelstein (Johns Hopkins University, USA) for kindly providing HCT116 Bax null cells.

This research was supported by the Basic Science Research Program, the National Research Foundation, Korea (2010-0009164), and a grant of the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (A062254).

References

[1] F. Gonzalvez, A. Ashkenazi, New insights into apoptosis signaling by Apo2L/TRAIL, Oncogene 29 (2010) 4752–4765.
[2] R.W. Johnstone, A.J. Frew, M.J. Smyth, The TRAIL apoptotic pathway in cancer onsetprogression and therapy, Nat. Rev. Cancer 8 (2008) 782–798.
[3] Y. Shi, Mechanisms of caspase activation and inhibition during apoptosis, Mol. Cell 9 (2002) 459–470.
[4] A.C. Bellail, L. Qi, P. Mulligan, V. Chhabra, C. Hao, TRAIL agonists on clinical trials for cancer therapy: the promises and the challenges, Rev. Recent Clin. Trials 4 (2009) 34–41.
[5] J.H. Stegehuis, L.H. de Wilt, E.G. de Vries, H.J. Groen, S. de Jong, F.A. Kruyt, TRAIL receptor targeting therapies for non-small cell lung cancer: current status and perspectives, Drug Resist. Updates 13 (2010) 2–15.
[6] C.M. Van Geelen, E.G. de Vries, S. de Jong, Lessons from TRAIL-resistance mechanisms in colorectal cancer cells: paving the road to patient-tailored therapy, Drug Resist. Updates 7 (2004) 345–358.
[7] J. Abdulghani, W.S. El-Deiry, TRAIL receptor signaling and therapeutics, Exp. Opin. Therapeut. Targets 14 (2010) 1091–1108.
[8] C.Y. Jin, C. Park, H.J. Hwang, G.Y. Kim, B.T. Choi, W.J. Kim, Y.H. Choi, Naringenin up-regulates the expression of death receptor 5 and enhances TRAIL-induced apoptosis in human lung cancer A549 cells, Mol. Nutr. Food Res. 55 (2011) 300–309.
[9] M. Lu, A. Strohecker, F. Chen, T. Kwan, J. Bosman, V.C. Jordan, V.L. Cryns, Aspirin sensitizes cancer cells to TRAIL-induced apoptosis by reducing survivin levels, Clin. Cancer Res. 14 (2008) 3168–3176.
[10] B. Oh, S. Park, J.H. Pak, I. Kim, Downregulation of Mcl-1 by daunorubicin pretreatment reverses resistance of breast cancer cells to TNF-related apoptosis-inducing ligand, Biochem. Biophys. Res. Commun. 422 (2012) 42–47.
[11] C. Mingorance, R. Rodriguez-Rodriguez, M.L. Justo, M.D. Herrera, M.A. de Sotomayor, Pharmacological effects and clinical applications of propionyl-L-carnitine, Nutr. Rev. 69 (2011) 279–290.
[12] B. Chang, M. Nishikawa, S. Nishiguchi, M. Inoue, L-Carnitine inhibits hepatocarcinogenesis via protection of mitochondria, Int. J. Cancer 113 (2005) 719–729.
[13] F. Ferrara, A. Bertelli, M. Falchi, Evaluation of carnitine, acetylcarnitine and isovalerylcarnitine on immune function and apoptosis, Drugs Exp. Clin. Res. 31 (2005) 109–114.
[14] S.Y. Choi, M.J. Kim, C.M. Kang, S. Bae, C.K. Cho, J.W. Soh, J.H. Kim, S. Kang, H.Y. Chung, Y.S. Lee, S.J. Lee, Activation of Bak and Bax through c-abl-protein kinase Cdelta-p38 MAPK signaling in response to ionizing radiation in human non-small cell lung cancer cells, J. Biol. Chem. 281 (2006) 7049–7059.
[15] H. Li, H. Zhu, C.J. Xu, J. Yuan, Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis, Cell 94 (1998) 491–501.
[16] M.J. Roy, S. Dionne, G. Marx, I. Qureshi, D. Sarma, E. Levy, E.G. Seidman, In vitro studies on the inhibition of colon cancer by butyrate and carnitine, Nutrition 11–12 (2009) 1193–1201.
[17] G. Vescovo, B. Ravara, V. Gobbo, M. Sandri, A. Angelini, M. Della Barbera, M. Dona, G. Peluso, M. Calvani, L. Mosconi, L. Dalla Libera, L-Carnitine: a potential treatment for blocking apoptosis and preventing skeletal muscle myopathy in heart failure, Am. J. Physiol. Cell Physiol. 283 (2002) C802–C810.
[18] E. Oyanagi, H. Yano, M. Uchida, K. Utsumi, J. Sasaki, Protective action of L-carnitine on cardiac mitochondrial function and structure against fatty acid stress, Biochem. Biophys. Res. Commun. 412 (2011) 61–67.
[19] U. Wenzel, A. Nickel, H. Daniel, Increased carnitine-dependent fatty acid uptake into mitochondria of human colon cancer cells induces apoptosis, J. Nutr. 135 (2005) 1510–1514.
[20] F. Ferrara, A. Bertelli, M. Falchi, Evaluation of carnitine, acetylcarnitine and isovalerylcarnitine on immune function and apoptosis, Drugs Exp. Clin. Res. 31 (2005) 109–114.
[21] J.P. Fan, H.S. Kim, G.D. Han, Induction of apoptosis by L-carnitine through regulation of two main pathways in Hepa1c1c 7 cells, Amino Acids 36 (2009) 365–372.
[22] J.Y. Kong, S.W. Rabkin, Palmitate-induced cardiac apoptosis is mediated through CPT-1 but not influenced by glucose and insulin, Am. J. Physiol. – Heart Circ. Physiol. 282 (2002) H717–725.
[23] D. Mahalingam, E. Szegezdi, M. Keane, S. de Jong, A. Samali, TRAIL receptor signalling and modulation: are we on the right TRAIL?, Cancer Treat Rev. 35 (2009) 280–288.
[24] Y. Li, H. Wang, Z. Wang, S. Makhija, D. Buchsbaum, A. LoBuglio, R. Kimberly, T. Zhou, Inducible resistance of tumor cells to tumor necrosis factor-related apoptosis-inducing ligand receptor 2-mediated apoptosis by generation of a blockade at the death domain function, Cancer Res. 66 (2006) 8508–8520.
[25] D. Hanahan, R.A. Weinberg, The hallmarks of cancer, Cell 100 (2000) 57–70.
[26] S. Hinz, A. Trauzold, L. Boenicke, C. Sandberg, S. Beckmann, E. Bayer, H. Walczak, H. Kalthoff, H. Ungefroren, Bcl-XL protects pancreatic adenocarcinoma cells against CD95- and TRAIL-receptor-mediated apoptosis, Oncogene 19 (2000) 5477–5486.
[27] S. Fulda, E. Meyer, K.M. Debatin, Inhibition of TRAIL-induced apoptosis by Bcl-2 overexpression, Oncogene 21 (2002) 2283–2294.
[28] A.D. Schimmer, K. Welsh, C. Pinilla, Z. Wang, M. Krajewska, M.J. Bonneau, I.M. Pedersen, S. Kitada, F.L. Scott, B. Bailly-Maitre, G. Glinsky, D. Scudiero, E.

Sausville, G. Salvesen, A. Nefzi, J.M. Ostresh, R.A. Houghten, J.C. Reed, Small-molecule antagonists of apoptosis suppressor XIAP exhibit broad antitumor activity, Cancer Cell 5 (2004) 25–35.
[29] M. Taniai, A. Grambihler, H. Higuchi, N. Werneburg, S.F. Bronk, D.J. Farrugia, S.H. Kaufmann, G.J. Gores, Mcl-1 mediates tumor necrosis factor-related apoptosis-inducing ligand resistance in human cholangiocarcinoma cells, Cancer Res. 64 (2004) 3517–3524.
[30] T. Wirth, F. Kühnel, B. Fleischmann-Mundt, N. Woller, M. Djojosubroto, K.L. Rudolph, M. Manns, L. Zender, S. Kubicka, Telomerase-dependent virotherapy overcomes resistance of hepatocellular carcinomas against chemotherapy and tumor necrosis factor-related apoptosis-inducing ligand by elimination of Mcl-1, Cancer Res. 65 (2005) 7393–7402.
[31] J. Adams, The development of proteasome inhibitors as anticancer drugs, Cancer Cell 5 (2004) 417–421.
[32] X.D. Zhang, S.K. Gillespie, J.M. Borrow, P. Hersey, The histone deacetylase inhibitor suberic bishydroxamate: a potential sensitizer of melanoma to TNF-related apoptosis-inducing ligand (TRAIL) induced apoptosis, Biochem. Pharmacol. 66 (2003) 1537–1545.
[33] E. Er, L. Oliver, P.F. Cartron, P. Juin, S. Manon, F.M. Vallette, Mitochondria as the target of the pro-apoptotic protein Bax, Biochim. Biophys. Acta 1757 (2006) 1301–1311.
[34] Y. Shen, E. White, P53-dependent apoptosis pathways, Adv. Cancer Res. 82 (2001) 55–84.
[35] A. Virmani, A. Koverech, S.F. Ali, Z.K. Binienda, Acetyl-L-carnitine modulates TP53 and IL10 Gene expression induced by 3-NPA evoked toxicity in PC12 cells, Curr. Neuropharmacol. 9 (2011) 195–199.
[36] M.M. Sayed-Ahmed, O.A. Al-Shabanah, M.M. Hafez, A.M. Aleisa, S.S. Al-Rejaie, Inhibition of gene expression of heart fatty acid binding protein and organic cation/carnitine transporter in doxorubicin cardiomyopathic rat model, Eur. J. Pharmacol. 640 (2010) 143–149.
[37] S.P. Cregan, J.G. MacLaurin, C.G. Craig, G.S. Robertson, D.W. Nicholson, D.S. Park, R.S. Slack, Bax-dependent caspase-3 activation is a key determinant in p53-induced apoptosis in neurons, J. Neurosci. 19 (1999) 7860–7869.
[38] L.F. Peterson, E. Mitrikeska, D. Giannola, Y. Lui, H. Sun, D. Bixby, S.N. Malek, N.J. Donato, S. Wang, M. Talpaz, P53 stabilization induces apoptosis in chronic myeloid leukemia blast crisis cells, Leukemia 25 (2011) 761–769.