Biochemical and Biophysical Research Communications 378 (2009) 21–26

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Caveolin-1 negatively regulates TRAIL-induced apoptosis in human hepatocarcinoma cells

Xiangxuan Zhao [a,b], Yong Liu [a,b], Qi Ma [a,b], Xiaohui Wang [a], Haijing Jin [a], Maryam Mehrpour [a,d], Quan Chen [a,c,*]

[a] The Joint Laboratory of Apoptosis and Cancer Biology, The State Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China
[b] The Graduate University of Chinese Academy of Sciences, Beijing 100049, China
[c] College of Life Science, Nankai University, Tianjin 300071, China
[d] INSERM U756, Faculté de pharmacie; Université Paris-Sud F-92296 Chatenay Malabry, France

ARTICLE INFO

Article history:
Received 6 October 2008
Available online 6 November 2008

Keywords:
Caveolin-1/Caveolae
Apoptosis
TRAIL
Death receptor-4

ABSTRACT

The tumor necrosis factor-related apoptosis-inducing ligand (TRAIL/Apo2L) offers promising therapeutic potential based on its ability to induce apoptosis in various cancer cell lines without obvious adverse effect to normal cells. However, the mechanism of the differential sensitivity towards TRAIL-induced apoptosis remains unclear. Here, we demonstrate that caveolin-1 directly regulated TRAIL-induced apoptosis in HepG2 cells. ShRNA-mediated caveolin knockdown sensitized TRAIL-induced apoptosis and disruption of caveolae structure by the cholesterol-extracting reagent, methyl-β-cyclodextrin (MCD), enhanced TRAIL-induced apoptosis. Over-expression of caveolin-1 partially blocked TRAIL-induced apoptosis. The engagement of TRAIL with its receptor DR4 reduced the localization of DR4 in caveolae and resulted in its internalization. Blockade of caveolae-mediated internalization of DR4 by filipin III effectively enhanced TRAIL-induced apoptosis. Collectively, our results reveal a new mechanism by which caveolin-1 negatively regulates TRAIL-induced apoptosis in human hepatocarcinoma cells.

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL, also called Apo2L) is a member of the tumor necrosis factor (TNF) ligand family that rapidly induces apoptosis in a variety of transformed cell lines [1]. TRAIL binding to two death receptors: TRAIL-R1 (DR4) or TRAIL-R2 (DR5) induces apoptosis as these receptors contain a functional death domain [2]. Unlike TNF-α or FASL, TRAIL suppresses the growth of TRAIL-sensitive human adenocarcinoma in mice and non-human primates without significant cytotoxic effects [3,4]. Although TRAIL is capable of inducing apoptosis in tumor cells of diverse origins, several studies have shown that most hepatoma cancer cell lines are resistant to its apoptotic effects, which suggests that TRAIL alone may be ineffective for cancer therapy.

Caveolae ('little caves') are flask-shaped lipid rafts, enriched in cholesterol, sphingolipids, and structural marker proteins termed caveolins [5]. Caveolins are highly conserved hairpin loop-shaped, oligomeric proteins of 22–24 kD, which play a critical role in normal caveolae function such as vesicular transportation and signal transduction [6]. It has been reported that caveolins are involved in regulation of G-proteins [7], protein kinase C [8], vascular endothelial growth factor receptor 2 [9], and platelet-derived growth factor receptor [10], which are consistently purified along with caveolae-derived membranes. Considerable experimental evidence indicates that Fas and TNF-R1 are either localized in or recruited to lipid rafts as an initial step necessary for the induction of apoptosis [11,12].

Interestingly, caveolin-1 has been implicated in the pathogenesis of oncogenic cell transformation, tumorigenesis, and metastasis (for review, see [13]). Caveolin-1 over-expression sensitizes fibroblast and bladder carcinoma cells to ceramide-induced death, through a PI3-kinase-dependent mechanism [14], and to cell death induced by staurosporine [15]. Cells expressing caveolin-1 were more susceptible to oxLDL-induced apoptosis [16]. Moreover, caveolin-1 over-expression in human prostate tumor cells renders them more resistant to apoptosis, while caveolin-1 down-regulation sensitizes these cells to apoptosis [17]. Taken together, the role of caveolin-1 in apoptosis remains controversial. Here we report that caveolin-1 negatively regulated TRAIL-induced apoptosis in HepG2 cells. Our findings suggest that caveolin-1 plays important roles in regulating tumor cell sensitivity to cancer drugs.

Materials and methods

Cell lines and reagents. Hepatoma HepG2 cells were cultured in 10% fetal bovine serum/RPMI 1640 supplemented with antibiotics (100 units/ml penicillin, and 100 mg/ml streptomycin) in a

* Corresponding author. Address. Nankai University, College of Life Science, Tianjin 300071, China.
E-mail address: chenquan@nankai.edu.cn (Q. Chen).

0006-291X/$ - see front matter © 2008 Published by Elsevier Inc.
doi:10.1016/j.bbrc.2008.10.123

humidified atmosphere with 5% CO_2 at 37 °C. Anti-caspase 8 mAb and anti-β-actin mAb were from Sigma-Aldrich; anti-caspase 3 rabbit pAb from Cell Signaling, Inc.; anti-caveolin-1 rabbit pAb, anti-TRAIL-Receptor-1 (DR4) goat pAb as well as the FITC-conjugated goat anti-rabbit second antibody from Santa Cruz Biotechnology; TRAIL (a truncated recombinant human TRAIL which contains 170 amino acid ($rhTRAIL_{170}$), Patent No: 02116263.8), was expressed in *Escherichia coli* and purified; filipin III and methyl-β-cyclodextrin were from Sigma–Aldrich; the SuperScript™ II reverse transcriptase kit, Lipofectamine 2000 and puromycin from Invitrogen.

Apoptosis assays. Apoptosis was assessed by detecting phosphatidylserine exposure on the cell membrane with Annexin V staining, as described previously [18]. Cells were simultaneously stained with Annexin V-FITC and PI. Comparative experiments were performed at the same time by bivariate flow-cytometry using a FACScan (BD) and analyzed with CellQuest software. Data were obtained from a cell population from which debris was gated out. Caspase 8 and 3 activation was determined by detecting the activated fragments by Western blotting. Pro-caspase 3 (37 kD) was cleaved into a 17 kD fragment but no cleaved band for pro-caspase 8 was detected during apoptosis.

Western blotting. Western blotting was performed according to our published method [18]. HepG2 cells were harvested and lysed in lysis buffer. Cellular protein was loaded and separated on sodium dodecyl sulfate polyacrylamide gel (SDS–PAGE) and transferred to a nitrocellulose membrane (GibcoBRL) by the standard electric transfer protocol. The membrane was blocked and probed with primary antibodies, then incubated with horseradish peroxidase-labeled second antibody (KPL Corp.). The membrane was then exposed to an enhanced chemiluminescent system (Pierce) and autoradiography was used to visualize immuno-reactive bands.

RNA interference (RNAi). The following oligonucleotides were cloned in to p-super-Babe-puro vector: 5′-(BglII)GATCCCCCAGGG CAACATCTACAAGCTTCAAGAGAGCTTGTAGATGTTGCCCTGTTTTTTA (HindIII)-3′. Empty vector was used as a control. HepG2 cell lines stably expressing the target gene were obtained by transfection with 2 μg psuper-Babe-puro vector containing the oligonucleotide and isolating stable clones after 5 days of selection with 2 mg/ml puromycin. The knockdown effect was measured by Western blotting.

Immunofluorescence staining. HepG2 cells plated onto 12-mm diameter round glass coverslips in 24-well plates were incubated with TRAIL for different times, then washed with PBS and fixed with paraformaldehyde. Fixed cells were permeabilized with 0.2% Triton X-100 and rinsed sequentially in PBS, NH_4Cl, and PBS. Cells were incubated with anti-caveolin-1 pAb. After three washes with PBS, cells were incubated with the FITC-conjugated goat anti-rabbit secondary antibody. Cells were washed three times with PBS. Cells on coverslips were mounted with anti-fade reagent onto glass slides and examined by fluorescence microscopy.

Extraction and purification of caveolae-enriched membrane fractions with alkaline carbonar. Caveolin-enriched membrane fractions were prepared as described previously with modification [19]. Briefly, normal or TRAIL-treated HepG2 cells were washed twice in PBS and scraped into sodium carbonate. The cell suspension was homogenized using a loose-fitting Dounce homogenizer, sheared in a polytron tissue grinder, and subjected to sonication using an ultrasonicator. The resulting homogenate was mixed with an equal volume of 90% (w/v) sucrose. The sample was then transferred to a 12 ml ultracentrifuge tube and overlaid with a discontinuous sucrose gradient 35% (w/v) sucrose and 5% (w/v) sucrose. The samples were subjected to centrifugation at 200,000*g* for 16–18 h at 4 °C. Twelve 1 ml fractions were collected from top to bot-

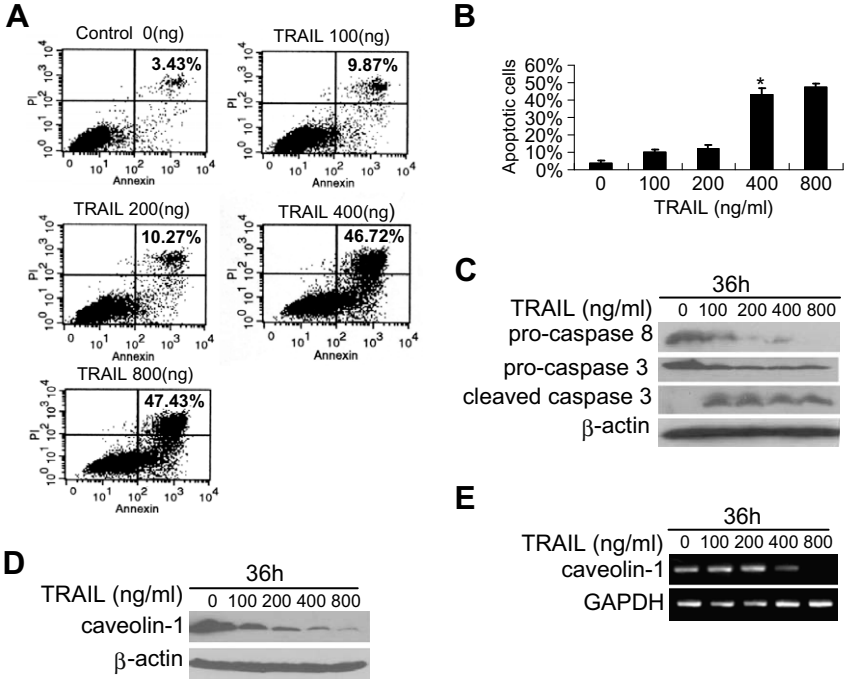

Fig. 1. TRAIL induces apoptosis and down-regulates caveolin expression in HepG2 cells. (A,B) Phosphatidylserine (PS) exposure. HepG2 cells cultured on six-well plates were incubated with different doses of TRAIL as indicated for 36 h. Apoptosis was evaluated by Annexin V staining and FACScan analysis. All data are expressed as mean ± SD from three individual experiments (B, $^*p < 0.05$). (C) Caspase activation. HepG2 cells exposed to different doses of TRAIL for 36 h were harvested and lysates were prepared and subjected to SDS–PAGE followed by Western blotting with antibodies against caspase 8 and caspase 3. The corresponding β-actin levels are shown as loading controls. (D) TRAIL-induced down-regulation of caveolin at protein level. HepG2 cells were incubated in the presence of TRAIL for 36 h. Total cell lysates were then prepared and analyzed for caveolin-1 expression by SDS–PAGE followed by Western blotting. (E) TRAIL-induced down-regulation of caveolin at mRNA level. Total mRNA was extracted from cells treated as in Fig. 1A, and the expression level of the caveolin-1 gene was evaluated using semi-quantitative RT-PCR. GAPDH served as a control for equal initial RNA amounts.

tom, and 30 μl aliquots of each fraction were subjected to SDS–PAGE and immunoblot analysis (detection of caveolin and actin). For co-localization and receptor shift experiments, the fractions were further purified by centrifugation at 300,000*g* with dilution using 2-fold MBS [20].

Results

TRAIL-induced apoptosis in HepG2 cells

HepG2 cells were incubated with TRAIL for 36 h and apoptosis was determined by Annexin V staining (measured by FACScan) (Fig. 1A and B) and by caspases activation (Fig. 1C). Cells displayed dose-dependent apoptotic sensitivity to TRAIL. Currently, the role of caveolin-1 in TRAIL-mediated apoptosis is not understood. To evaluate the correlation between caveolin-1 expression and TRAIL sensitivity, we first checked the expression level of caveolin-1 in HepG2 cells treated with TRAIL. Western blotting and semi-quantitative reverse transcription-PCR analysis showed that the caveolin-1 was decreased at both protein and mRNA level (Fig. 1D and E). These results indicated that TRAIL-mediated apoptosis is correlated with the down-regulation of caveolin-1 at both the mRNA and protein levels.

Caveolin-1 regulates TRAIL-induced apoptosis

To show that caveolin-1 was indeed functionally involved in the sensitivity of HepG2 cells to TRAIL-induced apoptosis, we further performed RNAi experiments to knockdown caveolin-1 expression. Western blotting analysis showed that the caveolin-1 level was significantly lower in cells harboring caveolin-1 RNAi vector than in cells transfected with the empty vector (Fig. 2A). We then analyzed the sensitivity of RNAi-transfected cells to TRAIL-mediated apoptosis. The data showed that caveolin-1 down-regulation sensitized HepG2 cells to TRAIL-mediated apoptosis ($^*p < 0.05$) (Fig. 2B).

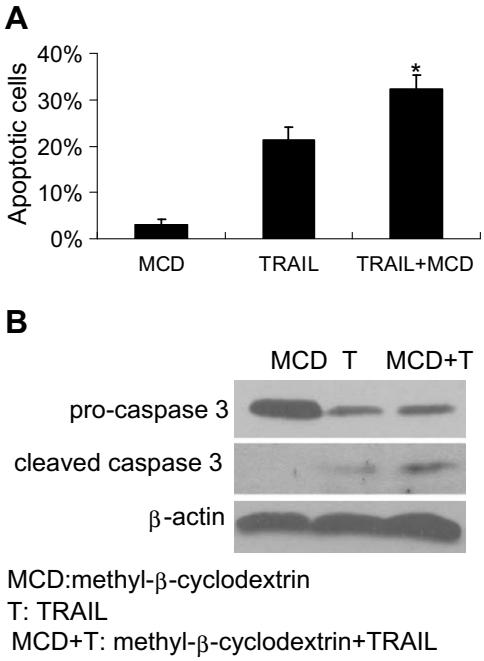

Fig. 3. Caveolae depletion by methyl-β-cyclodextrin sensitizes TRAIL-induced apoptosis. (A) Phosphatidylserine exposure. Cells were treated with 400 ng/ml TRAIL and/or 0.4 mM methyl-β-cyclodextrin (MCD), a caveolae depletion reagent, for 24 h. Apoptosis was evaluated as in Fig. 1A. All data are expressed as mean ± SD from three individual experiments ($^*p < 0.05$). (B) Caspase activation. Whole-cell lysates were subjected to SDS–PAGE, blotted and probed with antibodies recognizing caspase 3. The corresponding β-actin levels are shown as loading controls. The figure is representative of three similar independent experiments.

To determine the direct function of caveolin-1 in TRAIL-mediated apoptosis, we transiently transfected pcDNA3.1-caveolin-1 and de-

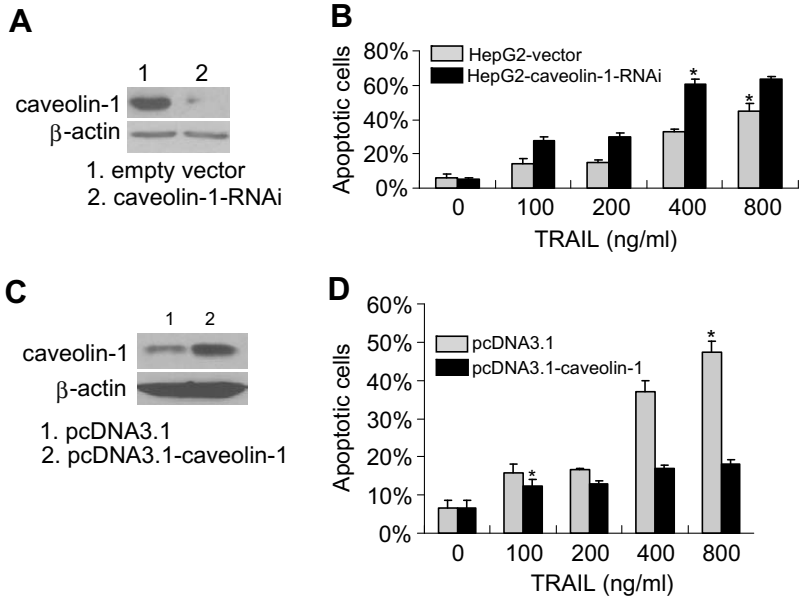

Fig. 2. Caveolin-1 regulates TRAIL-induced apoptosis. (A,B) Knockdown of caveolin by siRNA sensitizes cells towards apoptosis. Cells were transfected with 2 μg of caveolin-1 RNAi vector (p-super-Babe-puro-caveolin-1) or empty RNAi vector (p-super-Babe-puro). After 48 h, cell lysates were prepared and analyzed for caveolin-1 expression by SDS–PAGE followed by Western blotting. The corresponding β-actin levels are shown as loading controls. Cells harboring caveolin-1 RNAi vector or empty RNAi vector were exposed to the indicated concentration of TRAIL for 36 h. Apoptosis was evaluated as in Fig. 1A. All data are expressed as mean ± SD from three individual experiments (B, $^*p < 0.05$). (C,D) Caveolin over-expression blocks TRAIL-induced apoptosis. HepG2 cells transfected with pcDNA3.1-caveolin-1 or with pcDNA3.1 vector alone for 48 h were harvested and whole cell lysates were prepared and analyzed for caveolin-1 expression by SDS–PAGE followed by Western blotting with anti-caveolin-1 pAb. The corresponding β-actin levels are shown as loading controls. HepG2 Cells harboring pcDNA3.1-caveolin-1 or pcDNA3.1 empty vector alone were exposed to the indicated concentration of TRAIL for 36 h. Apoptosis was evaluated as in Fig. 1A. All data are expressed as mean ± SD from three individual experiments (D, $^*p < 0.05$).

tected caveolin-1 over-expression (Fig. 2C). Our results showed that cells transfected with empty pcDNA3.1 vector underwent more apoptosis than that of cells transfected with pcDNA3.1-caveolin-1 (Fig. 2D). Thus, over-expression of caveolin-1 prevented TRAIL-induced apoptosis in HepG2 cells. Taken together, these data suggest caveolin negatively regulate TRAIL-induced apoptosis in HepG2 cells.

Caveolae disruption sensitizes TRAIL-induced apoptosis

Caveolin is the key molecule that maintains the structure of caveolae, which may be crucial for the cell sensitivity to TRAIL. We thus anticipate that disruption of caveolae may sensitize the cells to TRAIL-induced apoptosis. To test whether TRAIL-mediated apoptosis requires caveolin-1 localization in caveolae, cells were exposed to MCD or cotreated with TRAIL for 24 h. We found that MCD enhanced TRAIL-induced apoptosis ($^{*}p < 0.05$) (Fig. 3A). Caspase activation results further supported the enhancing effects of MCD because TRAIL-induced caspase 3 processing was more marked in the presence of MCD (Fig. 3B, first band, third lane). These data suggest that depletion of caveolae cholesterol and the loss of compartmentalization of raft-associated molecules contributed to TRAIL-induced apoptosis.

Blockade of caveolae-mediated DR4 internalization sensitizes cells to TRAIL-induced apoptosis

Increasing evidences suggest that caveolin serves to gather the components of a signal transduction system in a spatially defined cell compartment and to prevent inappropriate activation. We hypothesized that engagement of TRAIL with its receptor could be affected by the caveolar structure within the plasma membrane. Thus, the plasma membranes of control and TRAIL-treated cells were collected and subjected to ultracentrifugation onto a linear sucrose gradient to collect 12 fractions. Each fraction was evaluated by Western blotting for the presence of DR4 and caveolin-1 (Fig. 4A). In intact cells, DR4 and caveolin-1 were expressed in fractions 5–6, which suggested that DR4 and caveolin-1 were co-localized in caveolar structures. We further used immunofluorescence staining to visualize caveolin-1 distribution or movement when treated with TRAIL. After 30 min incubation with TRAIL, caveolae-mediated internalization was able to be observed (Fig. 4B, white arrows showed concentrated and internalized caveolae). After 36 h, caveolin could not be detected because of caveolin down-regulation induced by TRAIL. Sucrose gradient fractions based on a carbonate scheme was collected and further purified and concentrated. Western blotting was carried out to analyze DR4 with a specific antibody directed against DR4. Our data showed that DR4 in caveolin-rich membrane domains decreased with TRAIL stimulation (Fig. 4C, top three, from 0, 6, and 36 h), while the caveolae-mediated endocytosis inhibitor filipin III blocked DR4 disappearance from caveolae (Fig. 4C, bottom two, 6 and 36 h). Furthermore, we asked whether this internalization contributed to the regulation of TRAIL-induced apoptosis. Our data showed that TRAIL-induced apoptosis in HepG2 cells was enhanced because of the blockade of DR4 internalization by filipin III ($^{*}p < 0.05$) (Fig. 4D). The above findings support the hypothesis that the TRAIL-induced apoptosis signaling pathway is regulated

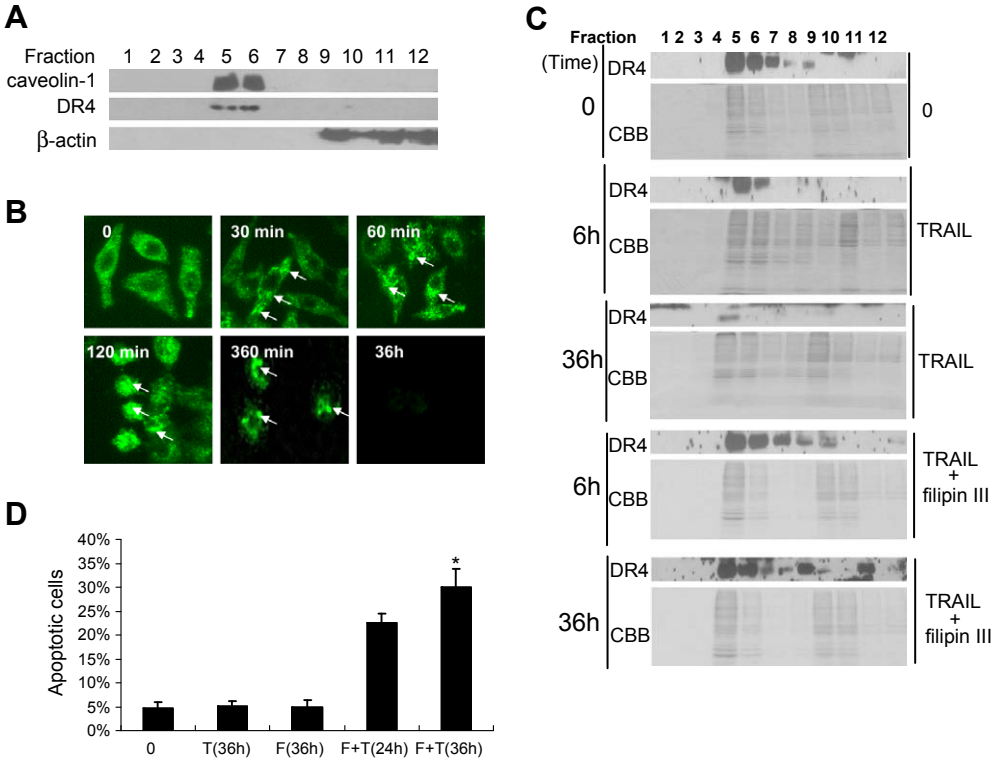

Fig. 4. Caveolae internalization correlates with TRAIL-induced apoptosis. (A) DR4 is co-localized with caveolin. HepG2 cells cultured on 5 × 75 cm^2 flasks were harvested and caveolae-enriched fractions were prepared. Fractions were analyzed by Western blotting for the presence of caveolin and DR4. (B) TRAIL induces caveolae/caveolin-1 internalization. HepG2 cells cultured on coverslips were treated with/without TRAIL (400 ng/ml) for indicated times. Cells were then immunostained with antibody that specifically recognizes caveolin-1. The bound caveolin-1 primary antibody was visualized with FITC-conjugated second antibody probes (green). (C) DR4 internalization. HepG2 cells treated with or without 400 ng/ml TRAIL in the presence or absence of filipin III for indicated times were harvested, lysed, and the lysates were treated as described in materials and methods. Western blotting analysis was carried out for DR4 movement from caveolae. Coomassie brilliant blue (CBB) stain was for comparison and served as equal loading controls. (D) Blockade of DR4 internalization sensitizes cells to TRAIL-induced apoptosis. HepG2 cells were treated with TRAIL (100 ng/ml) or not for indicated times in the presence or absence of filipin III (2 μg/ml). Apoptosis was determined as in Fig. 1A. All data are expressed as mean ± SD from three individual experiments (D, *p<0.05); F: filipin, T: TRAIL, and F + T: filipin + TRAIL.

by caveolae/caveolin through the formation of a special micro-environment.

Discussion

In this study we show that caveolin-1 negatively regulated apoptosis induced by TRAIL in hepatoma HepG2 cells. In addition, we find that cells' sensitivity to TRAIL-induced apoptosis is closely related to the integrity and normal movement of caveolae at the plasma membrane.

Since TRAIL induces apoptosis in various tumor cells, it is a promising candidate for cancer therapy [21]. However, in clinical trials many tumor cells were found to be insensitive to TRAIL-induced apoptosis. Interferon or UV is able to sensitize or enhance the ability of TRAIL to kill tumor cells [22]. Several groups have reported that caveolin/caveolae regulate the activation of other members of the TNF superfamily, including TNF-α and FasL [23]. Currently, the contribution of caveolin-1 to TRAIL-induced apoptosis is controversial. In our model, we first found that caveolin-1 was down-regulated both at the protein and mRNA levels, which suggested that caveolin-1 down-regulation was associated with the TRAIL cell death signal pathway. Using ectopic expression and gene silencing to pre-regulate caveolin-1 expression in normal HepG2 cells, we showed that it played an anti-apoptotic role. These data are consistent with the findings that caveolin-1 over-expression in Rat1A cells and human prostate cancer cells (LNCaP), or caveolin-1 up-regulation in androgen-insensitive LNCaP clones, renders these cells more resistant to apoptosis [24]. Whereas they contrast with the report that in fibroblasts caveolin-1 interacts with PI3-kinase, and caveolin-1 over-expression sensitizes to ceramide-induced death through a PI3-kinase-dependent mechanism [14]. A possible reason for the apparent incongruity of the pro-apoptotic or anti-apoptotic functions of caveolin-1 may be explained by cell-type specific effects: anti-apoptotic activity in some cancer cells and pro-apoptotic activity in other cell types. Alternatively, the disparate effects of caveolin-1 may be due to the use of different apoptosis inducers. Additional studies are necessary to clarify further the mechanism(s) by which caveolin is down-regulated by TRAIL.

Caveolae are cholesterol-enriched membrane microdomains. The sensitivity of caveolar architecture to cholesterol depletion and oxidation has been established, and cholesterol is necessary to stabilize caveolin oligomers [25]. Our demonstration that MCD, which destroys caveolar structure at the cell membrane, sensitized HepG2 cells to TRAIL-induced apoptosis provides further evidence that caveolae compartmentalize enzymatic reactions at the surface that are important for signaling. Compared to control cells treated with MCD alone, when incubated with TRAIL and MCD together, tumor cells become more sensitive to TRAIL stimulation. Our result was opposite to that obtained by disruption of caveolae using cholesterol-sequestering agents, which has been shown to block IL-6- and IGF-1-induced activation of the PI3-kinase/Akt signaling pathway [26]. The difference may lie in specific components and functions of caveolae.

Immunocytochemistry [27], cell fractionation [28], and immunoprecipitation [29] show that most cell surface proteins, including protein kinases, receptors, ion channels and growth factors, are located in caveolae. Using a canonical protocol we isolated caveolae structure for the HepG2 cell membrane. Our data showed that TRAIL-R1 (DR4) was partially localized in caveolae. Several groups reported from both morphologic [30] and biochemical [31] investigations that caveolae are involved in endocytosis. In caveolae/caveolin signal transduction research, the internalization of signal complexes under stress may be the mechanism for caveolae to sequester molecules, providing an opportunity for locally produced or imported molecules to modulate these signaling events [32]. Using immunostaining for caveolin, we showed that caveolae were internalized in cells treated with TRAIL along a time gradient. Our purification work provided evidence that TRAIL treatment induced DR4 internalization accompanied by caveolar structure on the cell membrane. With the specific inhibitor filipin III, we further showed that DR4 internalization induced by TRAIL was blocked, suggesting that DR4 internalization was caveolae-dependent. Our data support the idea that caveolae are the source of vesicles that move between the two surfaces of cells, and the process is inhibited by filipin III [31]. These data raise the possibility of an opposite caveolar movement compared to the topical application of vascular endothelial growth factor (VEGF), which rapidly induces the swelling and fusion of caveolae [33], indicating that the formation of caveolar channels is under hormonal control. We hypothesize that this type of internalization of signal molecules most likely has a variety of special uses in the cell. Interestingly, we found that pre-incubation of cells with filipin III enhanced apoptosis induced by TRAIL. This finding suggests that blockade of DR4 internalization by filipin III inhibited cells from sequestering the signal pathway. Undoubtedly, the ability of caveolin and caveolae to modulate signaling has important implications for the process of cell fate determination. These data suggest that with so many different signaling molecules in one location, caveolae are the logical place to look for signal integration.

Acknowledgment

This work was supported by grants from National Natural Science Foundation of China to Q.C. (No.35070419) and 973 programme project (2006CB910102 and 2007CB914800) from the Ministry of Science and Technology.

References

[1] S.A. Marsters, R.A. Pitti, J.P. Sheridan, A. Ashkenazi, Control of apoptosis signaling by Apo2 ligand, Recent Prog. Horm. Res. 54 (1999) 225–234.
[2] R.C. Humphreys, W. Halpern, Trail receptors: targets for cancer therapy, Adv. Exp. Med. Biol. 615 (2008) 127–158.
[3] B. Bonavida, C.P. Ng, A. Jazirehi, G. Schiller, Y. Mizutani, Selectivity of TRAIL-mediated apoptosis of cancer cells and synergy with drugs: the trail to non-toxic cancer therapeutics (review), Int. J. Oncol. 15 (1999) 793–802.
[4] O. Meurette, A. Fontaine, A. Rebillard, M.G. Le, T. Lamy, D. Lagadic-Gossmann, M.T. manche-Boitrel, Cytotoxicity of TRAIL/anticancer drug combinations in human normal cells, Ann. N.Y. Acad. Sci. 1090 (2006) 209–216.
[5] M. Stahlhut, K. Sandvig, D.B. van, Caveolae: uniform structures with multiple functions in signaling, cell growth, and cancer, Exp. Cell Res. 261 (2000) 111–118.
[6] P.W. Shaul, R.G. Anderson, Role of plasmalemmal caveolae in signal transduction, Am. J. Physiol. 275 (1998) L843–L851.
[7] M. Chun, U.K. Liyanage, M.P. Lisanti, H.F. Lodish, Signal transduction of a G protein-coupled receptor in caveolae: colocalization of endothelin and its receptor with caveolin, Proc. Natl. Acad. Sci. USA 91 (1994) 11728–11732.
[8] C. Mineo, Y.S. Ying, C. Chapline, S. Jaken, R.G. Anderson, Targeting of protein kinase Calpha to caveolae, J. Cell Biol. 141 (1998) 601–610.
[9] C.H. Cho, C.S. Lee, M. Chang, I.H. Jang, S.J. Kim, I. Hwang, S.H. Ryu, C.O. Lee, G.Y. Koh, Localization of VEGFR-2 and PLD2 in endothelial caveolae is involved in VEGF-induced phosphorylation of MEK and ERK, Am. J. Physiol. Heart Circ. Physiol. 286 (2004) H1881–H1888.
[10] P. Liu, Y. Ying, Y.G. Ko, R.G. Anderson, Localization of platelet-derived growth factor-stimulated phosphorylation cascade to caveolae, J. Biol. Chem. 271 (1996) 10299–10303.
[11] D. Lalor, P. Liu, J. Hayashi, Fas ligand is enriched in the caveolae membrane domains of thymic epithelial cells, Cell Immunol. 230 (2004) 10–16.
[12] Y.G. Ko, J.S. Lee, Y.S. Kang, J.H. Ahn, J.S. Seo, TNF-alpha-mediated apoptosis is initiated in caveolae-like domains, J. Immunol. 162 (1999) 7217–7223.
[13] T.M. Williams, M.P. Lisanti, Caveolin-1 in oncogenic transformation, cancer, and metastasis, Am. J. Physiol. Cell Physiol. 288 (2005) C494–C506.
[14] W. Zundel, L.M. Swiersz, A. Giaccia, Caveolin 1-mediated regulation of receptor tyrosine kinase-associated phosphatidylinositol 3-kinase activity by ceramide, Mol. Cell Biol. 20 (2000) 1507–1514.
[15] J. Liu, P. Lee, F. Galbiati, R.N. Kitsis, M.P. Lisanti, Caveolin-1 expression sensitizes fibroblastic and epithelial cells to apoptotic stimulation, Am. J. Physiol. Cell Physiol. 280 (2001) C823–C835.

[16] C. Ingueneau, U. Huynh-Do, J.C. Thiers, A. Negre-Salvayre, R. Salvayre, C. Vindis, Caveolin-1 sensitizes vascular smooth muscle cells to mildly oxidized LDL-induced apoptosis, Biochem. Biophys. Res. Commun. 369 (2008) 889–893.
[17] J.A. Karam, Y. Lotan, C.G. Roehrborn, R. Ashfaq, P.I. Karakiewicz, S.F. Shariat, Caveolin-1 overexpression is associated with aggressive prostate cancer recurrence, Prostate 67 (2007) 614–622.
[18] Q. Chen, B. Gong, A.S. Mahmoud-Ahmed, A. Zhou, E.D. Hsi, M. Hussein, A. Almasan, Apo2L/TRAIL and Bcl-2-related proteins regulate type I interferon-induced apoptosis in multiple myeloma, Blood 98 (2001) 2183–2192.
[19] S. Li, K.S. Song, M.P. Lisanti, Expression and characterization of recombinant caveolin. Purification by polyhistidine tagging and cholesterol-dependent incorporation into defined lipid membranes, J. Biol. Chem. 271 (1996) 568–573.
[20] M. Czarny, Y. Lavie, G. Fiucci, M. Liscovitch, Localization of phospholipase D in detergent-insoluble, caveolin-rich membrane domains. Modulation by caveolin-1 expression and caveolin-182-101, J. Biol. Chem. 274 (1999) 2717–2724.
[21] G. Fiorucci, S. Vannucchi, M.V. Chiantore, Z.A. Percario, E. Affabris, G. Romeo, TNF-related apoptosis-inducing ligand (TRAIL) as a pro-apoptotic signal transducer with cancer therapeutic potential, Curr. Pharm. Des. 11 (2005) 933–944.
[22] J.Z. Qin, P. Bacon, J. Panella, L.A. Sitailo, M.F. Denning, B.J. Nickoloff, Low-dose UV-radiation sensitizes keratinocytes to TRAIL-induced apoptosis, J. Cell Physiol. 200 (2004) 155–166.
[23] K. Ono, Y. Iwanaga, M. Hirayama, T. Kawamura, N. Sowa, K. Hasegawa, Contribution of caveolin-1 alpha and Akt to TNF-alpha-induced cell death, Am. J. Physiol. Lung Cell Mol. Physiol. 287 (2004) L201–L209.
[24] C.L. Tso, W.H. McBride, J. Sun, B. Patel, K.H. Tsui, S.H. Paik, B. Gitlitz, R. Caliliw, O.A. van, L. Wu, J. deKernion, A. Belldegrun, Androgen deprivation induces selective outgrowth of aggressive hormone-refractory prostate cancer clones expressing distinct cellular and molecular properties not present in parental androgen-dependent cancer cells, Cancer J. 6 (2000) 220–233.
[25] S. Monier, D.J. Dietzen, W.R. Hastings, D.M. Lublin, T.V. Kurzchalia, Oligomerization of VIP21-caveolin in vitro is stabilized by long chain fatty acylation or cholesterol, FEBS Lett. 388 (1996) 143–149.
[26] K. Podar, Y.T. Tai, C.E. Cole, T. Hideshima, M. Sattler, A. Hamblin, N. Mitsiades, R.L. Schlossman, F.E. Davies, G.J. Morgan, N.C. Munshi, D. Chauhan, K.C. Anderson, Essential role of caveolae in interleukin-6- and insulin-like growth factor I-triggered Akt-1-mediated survival of multiple myeloma cells, J. Biol. Chem. 278 (2003) 5794–5801.
[27] J. Liu, J.E. Garcia, T.A. Baramki, The difference in outcome of in-vitro culture of human testicular spermatozoa between obstructive and non-obstructive azoospermia, Hum. Reprod. 11 (1996) 1587–1588.
[28] G. Garcia-Cardena, P. Oh, J. Liu, J.E. Schnitzer, W.C. Sessa, Targeting of nitric oxide synthase to endothelial cell caveolae via palmitoylation: implications for nitric oxide signaling, Proc. Natl. Acad. Sci. USA 93 (1996) 6448–6453.
[29] J.M. Robinson, J.A. Badwey, The NADPH oxidase complex of phagocytic leukocytes: a biochemical and cytochemical view Histochem, Cell Biol. 103 (1995) 163–180.
[30] N. Simionescu, M. Simionescu, G.E. Palade, Permeability of muscle capillaries to exogenous myoglobin, J. Cell Biol. 57 (1973) 424–452.
[31] J.E. Schnitzer, P. Oh, E. Pinney, J. Allard, Filipin-sensitive caveolae-mediated transport in endothelium: reduced transcytosis, scavenger endocytosis, and capillary permeability of select macromolecules, J. Cell Biol. 127 (1994) 1217–1232.
[32] E. Botos, J. Klumperman, V. Oorschot, B. Igyarto, A. Magyar, M. Olah, A.L. Kiss, Caveolin-1 is transported to multivesicular bodies after albumin-induced endocytosis of caveolae in HepG2 cells, J. Cell Mol. Med. (2007).
[33] R.V. Stan, W.G. Roberts, D. Predescu, K. Ihida, L. Saucan, L. Ghitescu, G.E. Palade, Immunoisolation and partial characterization of endothelial plasmalemmal vesicles (caveolae), Mol. Biol. Cell 8 (1997) 595–605.