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LIGHT sensitizes IFNγ–mediated apoptosis of HT-29 human carcinoma cells through both death receptor and mitochondria pathways

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ABSTRACT

LIGHT [homologous to lymphotoxins, shows inducible expression, and competes with herpes simplex virus glycoprotein D for herpes virus entry mediator (HVEM/TR2)] is a new member of TNF superfamily. The HT-29 colon cancer cell line is the most sensitive to LIGHT-induced, IFNγ-mediated apoptosis among the cell lines we have examined so far. Besides downregulation of Bcl-X_L, upregulation of Bak, and activation of both PARP [poly (ADP-ribose) polymerase] and DFF45 (DNA fragmentation factor), LIGHT-induced, IFNγ-mediated apoptosis of HT-29 cells involves extensive caspase activation. Caspase-8 and caspase-9 activation, as shown by their cleavages appeared as early as 24 h after treatment, whereas caspase-3 and caspase-7 activation, as shown by their cleavages occurred after 72 h of LIGHT treatment. Caspase-3 inhibitor Z-DEVD-FMK (benzyloxycarbonyl-Asp-Glu-Val-Asp-fluoromethylketone) and a broad range caspase inhibitor Z-VAD-FMK (benzyloxycarbonyl-Val-Ala-Asp fluoromethylketone) were able to block LIGHT-induced, IFNγ-mediated apoptosis of HT-29 cells. The activity of caspase-3, which is one of the major executioner caspases, was found to be inhibited by both Z-DEVD-MFK and Z-VAD-FMK. These results suggest that LIGHT-induced, IFNγ-mediated apoptosis of HT-29 cells is caspase-dependent, and LIGHT signaling is mediated through both death receptor and mitochondria pathways.

Keywords: HT-29, LIGHT, apoptosis, Bcl-X_L, caspase.

INTRODUCTION

As a member of the TNF superfamily[1-4], LIGHT functions to induce apoptosis of cancer cells, especially in the presence of IFNγ[1-3, 5]. LIGHT causes growth arrest in RD (human rhabdomyosarcoma cell line) cells following developmental changes to smooth muscle cells, and it stimulates secretion of interleukin-8 and RANTES (regulated on activation normal T cell expressed and secreted) from the cells[6]. By blocking activation of both caspase-3 and caspase-8, LIGHT acts as an anti-apoptotic agent against TNFα-mediated live injury[7]. LIGHT is one of the CD28-independent co-stimulatory molecules in T cells; it is also required for dendritic cell-mediated allogenic T cell response in tumor and graft-versus-host disease models[8, 9]. Recently, studies on transgenic mice expressing recombinant LIGHT, mice administered soluble HVEM/TR2 proteins for blocking LIGHT activity, and the LIGHT knockout mouse have provided further proof that LIGHT is necessary for the expansion of T cells as well as playing an important role in T cell homeostasis[10-12]. It has been discovered that HIV 1 Nef simultaneously enhances surface expression of LIGHT, leading to enhanced cytokine activity, which in turn accelerates disease progression in infected individuals[13]. Also, it has been proposed that lymphotoxin (LT)/LIGHT axis controls microenvironments in the draining lymph nodes. These environments are critical in shaping the adjuvant-driven initiating events that impact the subsequent quality of the anti-collagen response in the later phase of collagen-induced arthritis[14].

LIGHT is the ligand for Herpes virus entry mediator (HVEM/TR2)[1, 2, 5, 15]. Our work showed that the apoptotic effect of LIGHT needed both lymphotoxin b receptor (LTbR) and HVEM/TR2[5]. Next, it was discovered that LTbR was sufficient for LIGHT-mediated

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Abbreviations: LTβR, lymphotoxin β receptor; LIGHT, homologous to lymphotoxins, shows inducible expression, and competes with herpes simplex virus glycoprotein D for herpes virus entry mediator (HVEM/TR2); TNF, tumor necrosis factor; IFN, interferon; PARP, poly (ADP-ribose) polymerase; AIF, apoptosis inducing factor; DFF, DNA fragmentation factor; TR, tumor necrosis factor receptor; Z-DEVD-FMK,benzyloxycarbonyl-Asp-Glu-Val-Asp-fluorome-thylketone; Z-VAD-FMK, benzyloxycarbonyl-Val-Ala-Asp-fluorome thylketone; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenylte-trazolium bromide.

apoptosis in HT-29 cells[3]. Besides binding to LTβR and HVEM/TR2, LIGHT also binds to TR6 [decoy receptor 3 (DcR3)], resulting in suppression of its apoptotic effect [16]. It has been suggested that LIGHT activates both pro-apoptotic and integrin-inducing pathways[3]. In the apoptotic cascade, LTβR recruits TNF receptor-associated factor-3 (TRAF3) in HT-29 cells[3]. In Hep3BT2 hepatocarcinoma cells, it was discovered that overexpression of anti-apoptosis Bcl-2 enhanced LIGHT- and IFNγ-mediated apoptosis through Bcl-2 cleavage. The pro-apoptosis function of the Bcl-2 cleavage fragment then triggered the apoptosis cascade by a process that did not necessarily require active caspase-3, which normally is a central and important effecter caspase in the apoptosis signal transduction pathways[17].

The HT29 colon cancer cell line expresses both LTβR and HVEM/TR2, and it is the most sensitive cell line to LIGHT-induced apoptosis among the cell lines we have examined[5]. Therefore, it is used as a model cell line to study LIGHT-induced, IFNγ-mediated apoptosis. So far, the upstream and downstream apoptosis signal transduction events of LIGHT-induced apoptosis remain unclear. Here we report that LIGHT induces apoptosis of HT29 cells in the presence of IFNγ through extensive activation of caspases and cleavage of both DFF45 and PARP.

MATERIALS AND METHODS

Cells and reagents

Human colon cancer cell line HT-29 was obtained from the National Cancer Institute (NCI, Federick, MD). It was maintained in Isokov's modified Eagles medium (Biofluids, Rockville) supplemented with 10% (v/v) heat-inactivated bovine serum (Gibco, BRL) plus 1% glutamine (Gibco, BRL) at 37°C in 5% (v/v) CO_2. The expression of apoptosis cascade components was detected using the following antibodies with immunoblotting: anti-Bcl-X_L mAb (Transduction Laboratories) for Bcl-X_L; anti-Bax rabbit polyclonal antibody (Upstate) for Bax; anti-Bid goat polyclonal antibody (Santa Cruz) for Bid; anti-phosphor-Bad (Ser112) mAb (Cell Signaling) for phosphor-Bad; anti-cpp32 rabbit polyclonal antibody (Pharmingen) for caspase-3; anti-caspase-7 rabbit polyclonal antibody, anti-caspase-8 mouse monoclonal antibody (Cell Signaling) for caspase-7, and -8; anti-caspase-9 rabbit polyclonal antibody (Santa Cruz) for caspase-9; anti-PARP rabbit polyclonal antibody (Roche Molecular Biochemicals) for PARP; cleaved DFF45 (D224) rabbit polyclonal antibody for cleaved DFF45 (Cell Signaling). IFNγ was purchased from Biosource International (Camarillo, CA). Caspase-3 inhibitor Z-DEVD-FMK and a broad range caspase inhibitor Z-VAD-FMK were purchased from Enzyme Systems Products (Livermore, CA).

Detection of apoptosis by flow cytometry

Cells undergoing apoptosis were detected by flow cytometry using a FACScan® (Becton Dickinson) with 488-nm laser line and analyzed using Cell Quest software. Phosphatidylserine exposed on the outside of the cells was determined by TACS™ Annexin V-FITC kit (Trevigen, Gaithersburg, MD). Briefly, cells were washed with cold PBS, pelleted and resuspended in 100 µl Annexin V-FITC diluted 1:100 in binding buffer (10 mM Hepes, 100 mM NaCl, 10 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$) containing propidium iodide (1:10). Cells were incubated for 10-15 min on ice, then an additional 400 µl binding buffer was added before FACScan® analysis. Annexin V-FITC fluorescence was detected in FL-1, and propidium iodide was detected in FL-2.

Measurement of cell growth

The survival rate of cells after treatment with LIGHT was determined using the MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium Bromide) method. Briefly, cells were seeded in 96-well flat bottom cell culture plates at a density of 5×10^4 cells/well. After treatment, 20 µl of 5 mg/ml MTT per well was added and incubated at 37°C for 4 h. Cells were then lysed by addition of 100 µl of DMSO per well and mixed well with a microplate shaker for about 5 min. The optical density of each sample was determined by measuring the absorbance at 570 nm versus 650 nm using an enzyme-linked immunosorbent assay reader (Molecular Device)

Immunoblot analysis

Cell lysate was prepared with lysis buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 1 %(v/v) Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 2 µg/ml leupeptin, and 2 µg/ml aprotinin). Equal amounts of protein were subjected to SDFS-PAGE electrophoresis, transferred onto nitrocellulose membrane (Hybond-C extra, Amersham Pharmacia Biotech), and reacted with appropriate antibodies in PBS containing 5% nonfat dry milk, 0.02% Tween 20. Blots were then incubated with horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence reagents subsequently (Amersham Pharmacia Biosciences, Picataway, NJ), followed by exposure to X-ray film (Kodak, Rochester, NY). Relative protein levels were quantified with the use of UN-SCAN-IT software (Silk Scientific Corp. Orem, Utah) on scanned films through digitization.

Measurement of caspase-3 activity

Caspase-3 activity was measured with ApoAlert® Caspase-3 Colorimetric Assay kit (Clontech, Palo Alto, CA). Briefly, 2×10^6 cells were collected and lysed with 50 ml chilled lysis buffer. Cell lysates were centrifuged in a microcentrifuge at maximum speed for 5 min at 4°C, 50 µl supernatants were transfered to 96 well plate, then 50 µl of 2×reaction buffer/DTT mix and 5 µl of 1 mM caspase-3 substrate (DEVD-pNA)were added to each reaction, incubated at 37°C for 1 h, and read at 405 nm in a microplate reader (Molecular Device). Final caspase-3 activity was calculated by dividing the net OD_{405nm} with the slope of a calibration curve obtained with different concentration of pNA.

RESULTS

LIGHT sensitizes IFNγ-mediated apoptosis of HT-29 cells

We have shown previously that HT-29 is the most susceptible cell line to LIGHT-induced, IFNγ-mediated growth inhibition[5,18,19]. To determine if the induction of

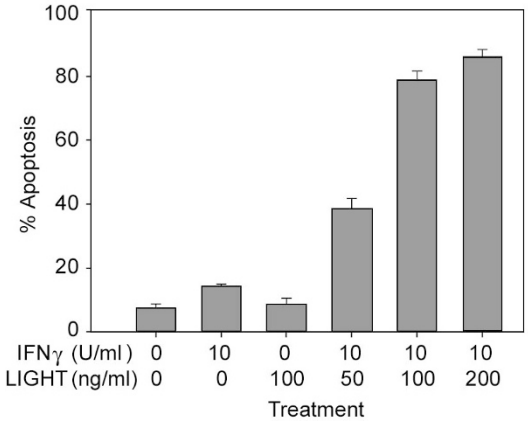

Fig 1. LIGHT-induced, IFNγ-mediated apoptosis of HT-29 cells. HT-29 cells were treated with different concentrations of LIGHT in the presence of IFNγ for 72 h. 1×10^6 cells were collected to conduct the Annexin V-Propidium Iodide double staining followed by flow cytometry analysis, as described in materials and methods. Numbers are mean values of three independent experiments±SD.

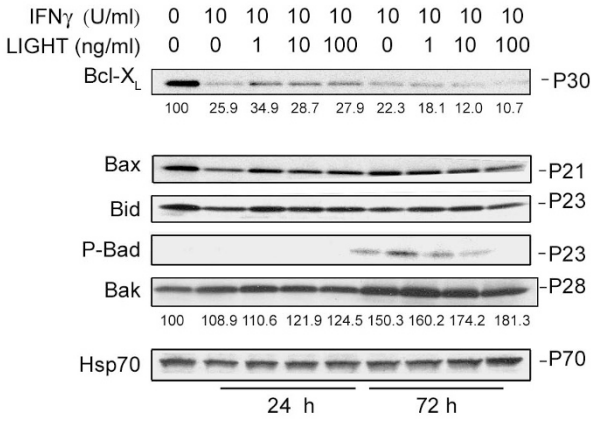

Fig 2. Alterations of Bcl-2 family members in LIGHT-induced apoptosis of HT-29 cells. HT-29 cells were treated with different concentration of LIGHT in the presence of IFNγ for various times. 20 μg cell lysates were subject to 4-20% gradient Tris-glycine gel electrophoresis followed by immunoblot analysis with Bcl-X_L, Bax, Bid, Phospho-(Ser112)-Bad, and Bak specific antibodies, respectively. Hsp70 probing confirms equal loading of the total protein. The percentage shows the relative protein expression level of Bcl-X_L and Bak compared with untreated cells. The figure is one representation of three independent experiments.

apoptosis contributes to this growth inhibition, we tested the treatment effects of LIGHT in combination with IFNγ in HT-29 cells with Annexin V-FITC and Propidium Iodide flow cytometry analysis, which specifically detects apoptosis. As seen in Fig 1, after 72 h of treatment, LIGHT alone (100 ng/ml) did not induce apoptosis of HT-29 cells (8.7%), and IFNγ (100 ng/ml) alone slightly induced apoptosis of HT-29 cells (14.2%), whereas combined use of both IFNγ (10 U/ml) and LIGHT (100 ng/ml) remarkably increased the apoptosis level (up to 79.1%) in a dose-dependent manner. This suggests that in combination with IFNγ, LIGHT sensitizes IFNγ-mediated apoptosis of HT-29 cells.

In order to determine if LIGHT can cause cell cycle arrest of HT-29 cells, cell cycle analysis was performed on HT-29 cells treated with different concentrations of LIGHT in the presence or absence of IFNγ. It was observed that there was no significant difference among untreated cells, cells treated with IFNγ alone, cells treated with LIGHT alone, and cells treated with both IFNγ and LIGHT in terms of their distribution in the G0-G1, G2-M, and S phases of the cell cycle. These findings demonstrate that LIGHT treatment does not cause cell cycle arrest of HT-29 cells; instead, it is a process of apoptosis.

LIGHT combined with IFNγ triggers downregulation of Bcl-X_L and upregulation of Bak

Overexpression of Bcl-2 and/or Bcl-X_L occurs in most cancer cells[20]. In order to elucidate whether LIGHT-induced apoptosis of HT-29 cells correlates with Bcl-2 and/or Bcl-X_L downregulation, Western blot analysis was performed to trace the changes of Bcl-2 family members upon treatment with LIGHT and IFNγ. Fig 2 shows the profile of most of the Bcl-2 family members upon treatment with LIGHT and IFNγ at various times. There was no Bcl-2 expression in HT-29 cells. It was observed that Bcl-X_L was downregulated (from 100% to 10.7%), and this downregulation was even more apparent after 72 h of treatment with 10 ng/ml of LIGHT. Bax and Bid levels remained unchanged, while Bak and Ser (112)-phospho-Bad levels were upregulated after 72 h of treatment at 10 ng/ml of LIGHT (Bak from 100% to 181.3%). These results suggest that LIGHT and IFNγ treatment triggers changes in the expression levels of Bcl-2 family members in HT-29 cells, among which, anti-apoptosis molecule Bcl-X_L downregulation and pro-apoptosis molecule Bak upregulation are the two major alterations.

Extensive caspase activation occurs during LIGHT-induced apoptosis of HT-29 cells

Recent discoveries have established that multiple distinct signaling pathways regulate apoptosis. Such pathways are activated in general by the formation of a death-inducing signaling complex (DISC). Activation of DISC results in the recruitment of inducer caspases (caspase-2, -8, -9, -12). These inducer caspases then amplify the apoptosis signal by cleavage and activation of effecter caspases (caspase-3, -6, -7), which execute apoptosis by degrading hundreds of regulatory proteins, resulting in activation of endo-

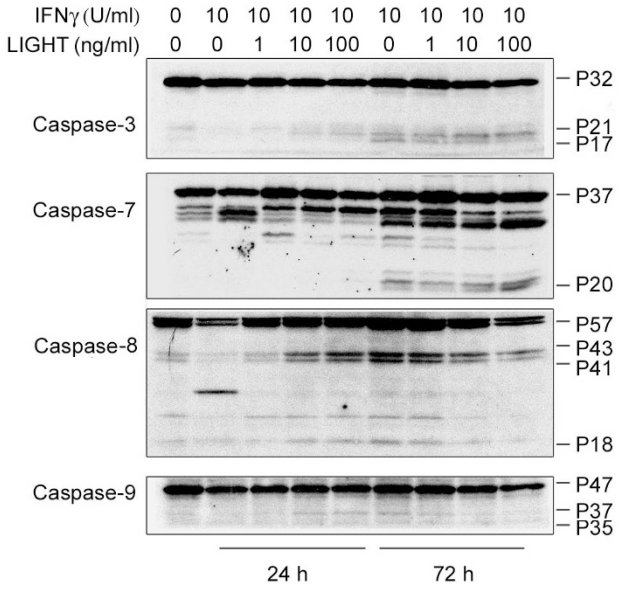

Fig 3. Extensive caspase activation occurred in LIGHT-induced apoptosis of HT-29 cells. HT-29 cells were treated with different concentration of LIGHT in the presence of IFNγ for various times. 20 μg cell lysates were subject to 4-20% gradient Tris-glycine gel electrophoresis followed by immunoblot with caspase-3, caspase-7, and caspase-8 and caspase-9 specific antibodies, respectively. The figure is one representation of three independent experiments.

nucleases and other proteins[21-23]. Thus, caspases are very important in the execution of apoptosis. To investigate if caspase activation is involved in LIGHT-induced, IFNγ-mediated apoptosis of HT-29 cells, expression of caspase-3, -7, -8, and -9 was analyzed using Western blot analysis. If activated, caspase-3 is cleaved into fragments P21 and P17, caspase-7 is cleaved into fragment P20, caspase-8 is cleaved into fragments P43/P41 and P18, and caspase-9 is cleaved into fragments P37 and P35. As illustrated in Fig 3, caspase-3 and caspase-7 were activated after 72 h of LIGHT treatment, since all the cleavage fragments of each caspase were observed at this time. In fact, the P21 fragment of caspase-3 and the P20 fragment of caspase-7 became more intense with increased LIGHT dosage. Caspase-8 and caspase-9 were activated as early as 24 h with 10 ng/ml of LIGHT as shown by the presence of their cleavage fragments. This activation decreased after 72 h treatment with 10 ng/ml of LIGHT. Activation of caspase-8 and caspase-9 occurred earlier than that of caspase-3 and caspase-7. These findings demonstrate that extensive caspase activation occurs during LIGHT-induced, IFNγ-mediated apoptosis of HT-29 cells. Activation of both caspase-8 and -9 indicates that LIGHT-signaling is through both the death receptor and mitochondria pathway.

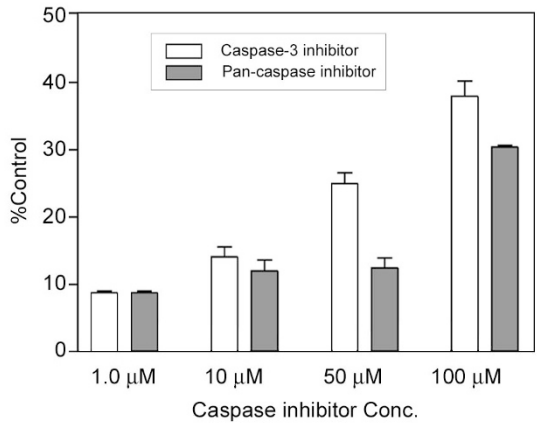

Fig 4. Activation of caspases is necessary for LIGHT-induced apoptosis of HT-29 cells. 2×10^4 HT-29 cells were treated with 100 ng/ml of LIGHT in the presence of 10 U/ml of IFNγ (final concentration) and different concentrations of Z-DEVD-FMK or Z-VAD-FMK for 72 h, cell survival rate was measured by the MTT method as described in the Materials and Methods. The figure is one representation of three independent experiments.

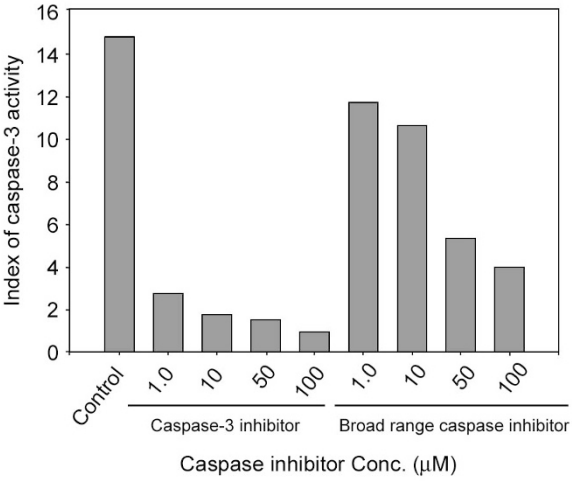

Fig 5. Caspase-3 is one of the most important caspases in LIGHT-induced apoptosis of HT-29 cells. 2×10^6 HT-29 cells were treated with the same conditions as in Fig 3 to detect caspase-3 activity using a colorimetric methods as described in the Materials and Methods. Data shown are representative of three independent experiments.

Blockade of caspase activity inhibits LIGHT-induced apoptosis of HT-29 cells

To further verify that caspase activation is necessary for LIGHT-induced apoptosis, HT-29 cells were treated with LIGHT in the presence of IFNγ and either a caspase-3 inhibitor, Z-DEVD-FMK or a broad range caspase inhibitor, Z-VAD-FMK. Cell survival rate was measured to see whether the antiproliferative effect of LIGHT combined

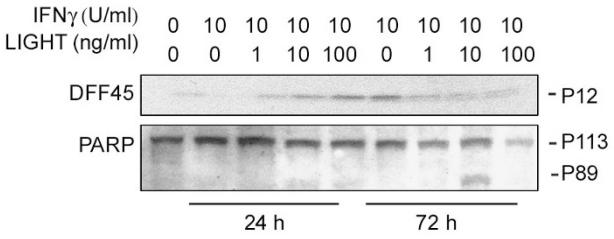

Fig 6. Cleavage of both DFF45 and PARP in LIGHT-induced apoptosis of HT-29 cells. HT-29 cells were treated with different concentration of LIGHT in the presence of IFNγ for various times. 20 μg cell lysates were subject to 4-20% gradient Tris-glycine gel electrophoresis followed by immunoblot analysis with a DFF45 fragment and a PARP specific antibody, respectively. The figure is one representation of three independent experiments.

with IFNγ was preventable. As shown in Fig 4, LIGHT-induced, IFNγ-mediated cell death was inhibited by both Z-DEVD-FMK and Z-VAD-FMK, since cell survival rate was increased. More cells were susceptible to the inhibition by Z-DEVD-FMK than that by Z-VAD-FMK. In order to verify that caspase activity plays a pivotal role in LIGHT-induced apoptosis of HT-29 cells, activity of one of the important caspases, caspase-3, was detected to determine whether such activity was related to LIGHT-induced apoptosis. As illustrated in Fig 5**,** caspase-3 activity decreased in the above-mentioned treatment of HT-29 cells, with cells more sensitive to treatment with Z-DEVD-FMK than Z-VAD-FMK. These results confirm that caspase activity is necessary for LIGHT-induced apoptosis of HT-29 cells, and caspase-3 may be the primary caspase involved. LIGHT-induced, IFNγ-mediated apoptosis of HT-29 cells is caspase-dependent.

Both DFF45 and PARP are cleaved in LIGHT-induced apoptosis

Caspase-3 and caspase-7 are two executive caspases which receive the apoptosis signal from mitochondria or death receptors, then the apoptosis signal is transmitted to DFF45 or PARP to act at the DNA level[24, 25]. Upon activation, DFF45 is cleaved into a P12 fragment and PARP is cleaved into fragments P89 and P24. As shown in Fig 6, DFF45 fragment P12 appeared as early as 24 h, then disappeared after 72 h with 10 ng/ml of LIGHT; Full-length PARP P119 existed in both untreated and all the treatment groups, but the intensity of this band weakened after 72 h of treatment, also P89 PARP cleavage part appeared with 10 ng/ml of LIGHT at 72 h of treatment. These observations suggest that the activation of both DFF45 and PARP is involved in LIGHT-induced apoptosis of HT-29 cells, but DFF45 might play a more important role.

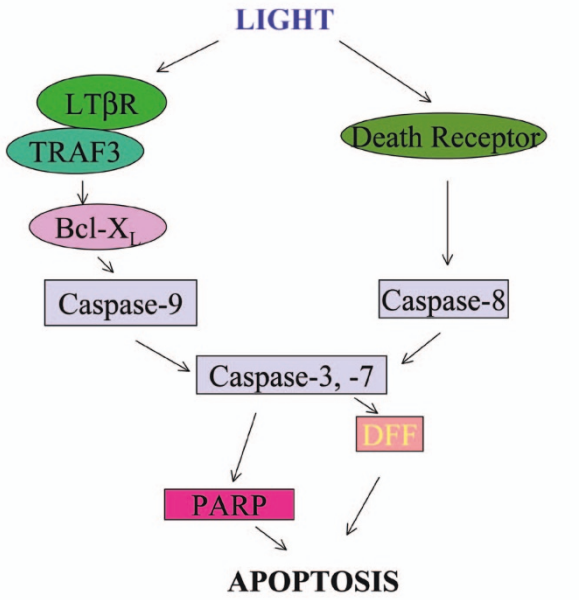

Fig 7. Death receptor and mitochondrial signal transduction pathways co-exist in HT-29 cells treated with LIGHT. LIGHT signaling triggers the death receptor pathway via activation of caspase-8. LIGHT signaling triggers the mitochondrial pathway through downregulation of Bcl-X_L as well as caspase-9 activation. Caspase-8 and caspase-9 then activate caspase-3 or caspase-7. The signal from caspase-3 or caspase-7 is first transmitted to DFF45, and then the signal is transmitted to PARP.

DISCUSSION

Treatment with both LIGHT and IFNγ downregulates Bcl-X_L. LIGHT signaling is through LTβR and TRAF3 [3]. These observations suggest that there is a link between LTβR and Bcl-X_L, indicating that the mitochondrial apoptosis signal transduction pathway is involved in LIGHT-induced, IFNγ-mediated apoptosis of HT-29 cells (Fig 7).

Our observation that Bax and Bid levels were unaltered indicates that they might not antagonize the anti-apoptotic effect of Bcl-X_L in HT-29 cells, as has been reported in other cells[20-24]. Bak was the only pro-apoptosis molecule which was upregulated in HT-29 cells treated by LIGHT and IFNγ, and it is worthy to note that the protein level of Bak was the highest compared with the other Bcl-2 family members (Bcl-X_L, Bax, Bid, P-Bad). Increased expression of Bak might be enough to antagonize the anti-apoptotic effect of anti-apoptosis Bcl-X_L and upregulated Ser (112)-phosphor-Bad. It has been shown that Bak is the most important pro-apoptosis Bcl-2 family member, whose function is to determine whether or not apoptosis proceeds in the cell[26-29]. This observation correlates with the observation that Bid expression levels remained

unaltered, while phospho-(Ser112)-Bad and Bak were upregulated in MDA-MB-231 breast carcinoma cells treated with LIGHT in the presence of IFNγ[19].

Three major apoptotic pathways originating from three separate subcellular compartments have been identified: the death receptor-mediated pathway, the mitochondria pathway, and the endoplasmic reticulum pathway[23, 30]. LIGHT-induced apoptosis of HT-29 cells is apparently involved in the death receptor pathway and the mitochondria pathway, because activation of caspase-8[31-34] and alteration of the expression levels of Bcl-2 family members were observed[35, 36]. Because we observed activation of caspase-9 and no change in the expression level of Apaf-1 and AIF (data not shown), we predict that other factors like Smac/DIABLO[37-39] might be the effectors, rather than cytochrome c[40-42] and AIF[43-45]. Also, since caspase-8 and caspase-9 were activated at approximately the same time, the death receptor pathway and the mitochondrial pathway are possibly parallel pathways in HT-29 cells treated with LIGHT. Besides LTβR and HVEM/TR2, it is possible that LIGHT could bind to the death receptors (Fig 7), in a manner similar to that of TRAIL, because its protein sequence shares some homology with other TNF members[46-50]. It remains unclear how LIGHT signaling is involved downstream of these pathways, especially from TRAF3 to caspase-8, and from TRAF3 to mitochondria, leading to the altered expression of some Bcl-2 family members. The other important issue is that expression of TR6[16] must have been depressed by LIGHT induced, IFNγ-mediated apoptosis, but whether TR6 was downregulated and how this signal was transmitted to the apoptosis cascade is worthy of further investigation.

The broad range caspase inhibitor, the caspase-1 inhibitor, and the caspase-3 inhibitor do not completely block LIGHT/IFNγ induced apoptosis in Hep3BT2 cells, so it was proposed that a caspase-independent apoptosis pathway might exist through which reactive oxygen species (ROS) and other inducers could bypass alteration of mitochondria[17], as reported by others[51-53]. In LIGHT-induced apoptosis of HT-29 cells, altered expression of Bcl-2 family members and extensive caspase activation of caspases-3, -7, -8, and -9, were observed. Furthermore, LIGHT-induced apoptosis of HT-29 cells was blocked by caspase inhibitors, especially caspase-3 inhibitor. These results support our conclusion that LIGHT-induced, IFNγ-mediated apoptosis of HT-29 cells is caspase-dependent. This observation differs from those findings observed in LIGHT-induced, IFNγ-mediated apoptosis of MDA-MB-231 breast cancer cells. In these cells, almost all the caspase activation was observed, but cell growth inhibition was not completely blocked by either of these two caspase inhibitors[19]. Therefore, caspase-dependency might be the reason HT-29 cells can reach higher rate of apoptosis than MDA-MB-231 cells.

LIGHT alone does not induce apoptosis in HT-29 cells. It must get help from IFNγ. IFNγ is a pleiotropic cytokine; it can both inhibit and stimulate cell growth[54]. It has been reported that IFNγ-induced apoptosis occurs through Fas/CD95[55, 56]. Therefore, LIGHT sensitizing IFNγ-mediated apoptosis of HT-29 cells is probably a synergistic cytotoxic effect. In another report, LIGHT did not induce apoptosis in the presence of IFNγ (shown by Bcl-2 down-regulation) of STAT1 deficient fibrosarcoma cells U3A, but did induce apoptosis of STAT1 knock-in cells U3A1-1 (Zhang et al; unpublished data). These results are consistent with the observation that activation of the STAT signaling pathway causes apoptosis[57]. That is, IFNγ signaling takes part in the apoptosis of HT-29 cells. The manner by which LIGHT and IFNγ cross-talk between each other to activate downstream apoptosis pathway is yet unknown.

In summary, LIGHT signaling in HT-29 cells is involved in two parallel pathways: death receptor and mitochondria. It is a caspase-dependent process, and DFF45 is used as a rapid executioner to damage DNA.

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