Expression of TNF-related apoptosis-inducing Ligand receptors and antitumor tumor effects of TNF-related apoptosis-inducing Ligand in human hepatocellular carcinoma

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Abstract

AIM: To investigate the expression of TNF-related apoptosis-inducing Ligand (TRAIL) receptors and antitumor effects of TRAIL in hepatocellular carcinoma (HCC).

METHODS: Expression of TRAIL receptors was determined in 60 HCC tissues, 20 normal liver samples and two HCC cell lines (HepG2 and SMMC-7721). The effects of TRAIL on promoting apoptosis in HCC cell lines were analyzed after the cells were exposed to the recombinant TRAIL protein, as well as transfected with TRAIL-expression construct. In vivo effects of TRAIL on tumor growth were investigated by using nude mice HCC model of hepG2.

RESULTS: Both death receptors were expressed in all HCC tissues and normal hepatic samples. In contrast, 54 HCC tissues did not express DcR1 and 25 did not express DcR2. Both DcR were detectable in all of the normal liver tissues. The expression patterns of DR and DcR in HCC samples (higher DR expression level and lower DcR expression level) were quite different from those in normal tissue. DR5, DR4, and DcR2 expressed in both cell lines, while no DcR1 expression was detected. Recombinant TRAIL alone was found to have a slight activity as it killed a maximum of 15% of HCC cells within 24 h. Transfection of the TRAIL cDNA failed to induce extensive apoptosis in HCC lines. In vivo administration of TRAIL gene could not inhibit tumor growth in nude mice HCC model. However, chemotherapeutic agents or anticancer cytokines dramatically augmented TRAIL-induced apoptosis in HCC cell lines.

CONCLUSION: Loss of DcR (especially DcR1) in HCC may contribute to antitumor effects of TRAIL to HCC. HCC is insensitive towards TRAIL-mediated apoptosis, suggesting that the presence of mediators can inhibit the TRAIL cell-death-inducing pathway in HCC. TRAIL and chemotherapeutic agents or anticancer cytokines combination may be a novel strategy for the treatment of HCC.

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INTRODUCTION

Apoptosis, the process of programmed cell death, is a fundamental mechanism in developmental and homeostatic maintenance of complex biological systems. Maladjustment or disturbance in apoptotic process will result in transformation and provide a growth advantage to transformed cells. Members of the tumor necrosis factor (TNF) superfamily contribute to a variety of cell biological functions, including cellular activation, proliferation and death, by interaction with their corresponding receptors. TNF and fasL have been focused on because of their antitumor activity, whereas TNF and fas ligand were unlikely to provide useful target neoplastic elimination of tumors cells for two reasons. One is that many tumor cells are resistant to FasL or TNF-mediated cell death, as shown in the present studies, the other is that systemical administration of TNF and FasL is limited by their acute cytotoxic effects on normal tissues in vivo, thereby limiting their widespread use in the treatment of cancer.

TNF-related apoptosis-inducing ligand (TRAIL), another TNF superfamily member, is a promising cancer therapeutic agent. TRAIL appears to specifically kill transformed cells, and spare most normal cells. TRAIL induces apoptotic death on binding to either of two proapoptotic TRAIL receptors, DR5 or DR4. Normal cells are believed to be resistant to TRAIL because of expressing higher levels of TRAIL decoy receptors DcR1 or DcR2 on their cell surface.

A recent study reported that chemotherapeutic agents could augment TRAIL-induced apoptosis in human hepatocellular carcinoma cell lines, indicating that TRAIL may have therapeutic potential in the treatment of human HCC. In the present study, we investigated the expression of TRAIL receptors and antitumor effects of TRAIL in human HCC, in order to find an effective treatment of HCC.

MATERIALS AND METHODS

HCC and control liver Tissues

Sixty surgically resected specimens employed in this study were obtained from patients with HCC who had undergone potentially curative tumor resection at the Hepatic Surgery Center, Tongji Hospital during January, 2000-December, 2000. All HCC tissues were pathologically confirmed. Twenty normal liver samples obtained from patients with benign tumor were used as control at the same time. We obtained informed consent from all patients. Resected tissues were frozen immediately at -70°C. All cases were selected on the basis of availability of frozen materials for study and in the absence of extensive tumor necrosis. Materials were composed of 5 cases of grade I, 23 cases of grade II, 27 cases of grade III and 5 cases of grade IV according to TNM system (1987). The tumor
lesions analyzed here including 35 poor differentiations, 15 moderate differentiations and 10 well differentiations. There were 53 males and 7 females, and the age was from 18 to 75 years with an average of 45.8 years (s, 13.5 years), HBsAg positive 55 and negative 5. Routinely processed 4 % paraformaldehyde-fixed, paraffin-embedded blocks containing principal tumor were selected. Serial sections of 5 µm thickness were prepared from the cut surface of blocks at the maximum cross-section of tumor.

**Cell lines and cell culture**

Human HCC cell lines (HepG2, SMMC-7721), Jurkat T-cell line and human colangiocarcinoma cell line QBC939 were purchased from ATCC. Cells were cultured in DMEM supplemented with 1 % penicillin/streptomycin and 10 % heat-inactivated fetal calf serum in a 5 % CO2 incubator at 37 °C.

**Detection of TRAIL-R**

The expression of TRAILR1, TRAILR2, TRAILR3 and TRAILR4 in HCC cell lines and human HCC tissues was detected in situ hybridization. Digoxigenin-labeled antisense and sense TRAIL-R probes were purchased from Boster Biotechnology Inc. In situ mRNA expressions were performed according to the manufacturer’s protocol. Deseaparaffinized, rehydrated tissue sections (5 µm) on silane-coated slides were permeabilized by incubation in proteinase 75 (20 µg/ml) for 30 min at 37 °C and acetylated in PBS containing 0.25 % acetic anhydride and 0.1 M triethanolamine for 10 min at room temperature. Prehybridization was carried out in 50 % formamide, 4×SSC, 1×Denhardt’s solution, 125 µg/ml tRNA, and 100 µg/ml freshly denatured salmon sperm DNA for 2-4 h at 42 °C. Hybridization was performed using prehybridization solution containing denatured TRAILR antisense or sense probes (50 ng/slide) for 36 h at 42 °C and followed by washes at 52 °C with different solutions (2×SSC and 50 % formamide, 30 min; 1×SSC and 50 % formamide, 30 min; 0.5×SSC and 50 % formamide, 30 min). After that, the sections were incubated in turn in buffer 1 [150 mM NaCl and 100 mM Tris-HCl (pH 7.5)] containing 5 % BSA and 0.3 % Triton X-100 for 30 min and in horseradish peroxidase-conjugated antidigoxigenin antibody diluted 1:500 with buffer 1 containing 5 % BSA and 0.3 % Triton X-100 for 2 h at room temperature. After washed in buffer 1, the sections were immersed briefly in buffer 2 [100 mM NaCl, 50 mM MgCl2, and 100 mM Tris-HCl (pH 9.5)] and incubated in buffer 2 containing 0.025 % dianisobenzenide and 0.02 % H2O2 for 15 min at 4 °C. Finally, the sections were immersed in 10 mM Tris-HCl, 1 mM EDTA buffer (pH 8.0), rinsed in water, and mounted with glycerol mountant.

**In vitro effects of TRAIL on cellular apoptosis of HCC**

HCC cells were seeded into 96-well microtiter plates with 5x103 cells per well and cultured in DMEM supplemented with 10 % fetal calf serum at 37 °C under a humidified atmosphere of 5 % CO2 for 24 h when the cells were in the exponential phase of growth. After treated with TRAIL at the indicated concentration (1, 10, 100, 1000 ng/ml), the cell viability was assessed with MTT method and the absorbance was measured at 490 nm with a microtiter plate reader. Cell death was estimated with the following formula.

\[
\% \text{ specific death} = \frac{A(\text{untreated cells}) - A(\text{treated cells})}{A(\text{untreated cells})} \times 100
\]

Results were derived from 3 individual experiments. Each experimental condition was repeated at least in sextuplicate wells for each experiment. We used the same method to detect the effects of TRAIL on Jurkat T-cell line and human colangiocarcinoma cell line QBC939.

**Construction of TRAIL expression plasmids**

The sequence corresponding to the C-terminus extracellular region of 114-281 amino acid (aa) of TRAIL was amplified by PCR, and subcloned into the EcoRV/EcoRI site of expression vector pIRES-EGFP. Following DNA plasmid transfection with superfect reagent for 72 hours, cells were evaluated for apoptosis. The expression of cellular TRAIL protein was verified with Western blot. Briefly, cells were collected by centrifugation at 2 000 g for 10 minutes, cells lysed with lysis cell solution (50 mmol/L Tris-cl, 150 mmol/L NaCl, 0.02 % sodium azide, 0.1 % SDS, 100 µg/ml PMSF, 1 µg/ml aprotinin, 1 % NP-40, 0.5 % deoxyxysodium cholate), protein in the supernatants was separated by 12 % SDS-PAGE and transferred by electroblotting to nitrocellulose membranes. The membranes were blocked by TBST (TBS-Tween) with 5 % non-fat milk, and then incubated with primary antibody (anti-hTRAILmAb) in TBST with 5 % non-fat milk overnight. Proteins were detected with horseradish peroxidase-conjugated secondary antibody and performed chemiluminescence according to the manufacturer’s instructions. MTT assay was used to measure cell viability. The tumor cells were infected with pIRES-EGFP-null as a control.

**Flow cytometry**

Collected cells were washed with PBS, fixed in 70 % ethanol, digested with RNase A (10 mg/L) in PBS for 30 min and then stained with propidium iodide (PI), and analyzed on a FACScan.

**Gene therapy of experimental nude mice subcutaneous hepatocellular carcinoma by direct intratumoral injection of TRAIL gene**

5x102 HepG2 cells were inoculated subcutaneously into the back of nude mice. One cm tumors were formed in nude mice about ten days after inoculation. Plasmid carrying TRAIL gene was delivered into the tumor by intra-tumor injection (n=10). pIRES-EGFP-null injection as a control (n=10). RT-PCR was adopted to examine the expression of TRAIL gene in tumor. The expression of TRAIL protein was detected by Western blotting. The anti-tumor effect of gene therapy was evaluated according to the sizes of tumors for 4 weeks. Therapeutic effects of TRAIL gene on experimental cholangiocarcinoma induced in nude mice were detected using the same method.

**Treatment of HCC cell lines in combination with TRAIL and IFN-γ, IL-2 or chemotherapy drug**

HCC cells exposed to IL-2 (different concentrations) combined with recombinant TRAIL protein (50 ng/ml) for 72 h were compared with treatment of TRAIL alone. In the same time, the effect of TRAIL in combination with IFN-γ or chemotherapy drugs (such as mitomycin, 5-fluorouracil) was investigated. Cell viability was examined by MTT assay.

**Statistical analysis of data**

Quantitative comparisons were performed by Student t test or the chi-square and Fisher exact tests. All statistical analyses were performed using SAS software. Statistical significance was set at 0.05.

**RESULTS**

**Expression of TRAIL-R in HCC cell lines and tissues**

DR5 and DR4 were present in all HCC tissue as well as normal hepatic tissues. In contrast, 54 tumors did not express DcR1 and 25 tumors did not express DcR2, but DcR1 and DcR2 were detected in all of the normal liver tissues. The expression patterns of DR and DcR in HCC samples (higher DR expression
level and lower DcR expression level) were quite different from those in normal tissue. DR5, DR4, and DcR2 were expressed, but DcR1 did not express in both cell lines. Expression of TRAIL-R was located mainly in the cytoplasm (Figure 1).

**Figure 1** Expression of TRAIL-R in human HCC tissues and HCC cell lines by in situ hybridization. Stronger expression of DR5 (A and D) and DR4 (B and E) as well as negative expression of DcR1 (C and F) in both human HCC tissues and HCC cell lines. Note that TRAIL-R staining was found mainly in the cytoplasm and membrane.

**Figure 2** Construction of pIRES-EGFP-TRAIL. A: the C-terminus extracellular region of 114-281 amino acid (aa) was amplified by PCR, including EcoRV/EcoRI at two end, 523bp. B: TRAIL expression plasmids pIRES-EGFP-TRAIL. C: The cellular proteins were separated by SDS-PAGE. D: Western blot identified expression of TRAIL, negative expression of TRAIL in control.
Production of TRAIL protein
TRAIL expression was assayed by Western blotting as shown, a 19.6 KD band sTRAIL monomers. In contrast, no corresponding bands were present in the negative control. These results demonstrated that the transfection of pIRES-EGFP-TRAIL resulted in transgene expression in human liver cancer cell lines (Figure 2).

Resistance of human HCC cells to both TRAIL and pIRES-EGFP-TRAIL transfection
TRAIL protein alone failed to induce significant apoptosis in both HCC cell lines, even at a dose up to 100 ng/ml, it killed only about 10 % of HCC cells within 24 h, compared with 70 % of Jurkat cells and about 50 % of cholangiocarcinoma cell line QBC939. Production of TRAIL following pIRES-EGFP-TRAIL transfection also failed to lead to tumor cell death.

HCC cell lines were adequately transfected with pIRES-EGFP-TRAIL. Minimal cell death of HepG2 cells was observed upon infection with pIRES-EGFP-TRAIL. Analysis of TRAIL protein production by Western blot revealed detectable levels in HepG2 cell lysed by 6h post infection, with levels increasing over the entire time course. These results demonstrated that tumor cells infected with pIRES-EGFP-TRAIL could produce TRAIL protein, however failed to lead to their extensive death (Figure 3).

Effects of gene therapy on experimental hepatocellular carcinoma
Direct intra-tumor injection of pIRES-EGFP carrying TRAIL gene was performed on experimental hepatocellular carcinoma induced in nude mice. RT-PCR was adopted to examine the expression of TRAIL gene in nude mice tumor, the anti-tumoral...
effect of gene therapy was evaluated according to the sizes of the tumors (as shown by Figure 4). Ten days after transfection of TRAIL gene, the expression of TRAIL gene in nude mice tumor was relatively strong, the expression of TRAIL transgene in tissue near the tumor was weaker and that in normal liver tissue was negative.

However, the tumor sizes were slightly less than those in control group (as shown in Table 1). TRAIL gene intra-tumor injection could lead to effective gene delivery and gene expression but had limited effect on experimental HCC in nude mice.

Table 1 Antitumor effects of pIRES-EGFP-TRAIL transfection on tumor growth in nude mice (tumor sizes, cm)

| Target cell | pIRES-EGFP-TRAIL (n=10) | pIRES-EGFP-null (n=10) | P |
|-------------|-------------------------|------------------------|---|
| HepG2       | 1.05±0.39               | 1.30±0.27              | 0.174 |
| QBC939      | 0.85±0.23               | 0.32±0.15              | 0.023 |

Synergistic induction of apoptosis by combination of TRAIL and chemotherapeutic drugs

We incubated HCC cells with mitomycin (0.001 mg/ml) or 5-fluorouracil (5-Fu) (0.025 mg/ml) in combination with TRAIL (50 ng/ml). Cell viability was examined after 24-72 h. Cytotoxicity of the combination of TRAIL and chemotherapeutic agents was compared with either agent alone (Figure 5). Sensitization of HCC cells to TRAIL-inducing apoptosis was independent of p53 status because both HepG2 and SMMC-7721 were similarly sensitized to TRAIL in combination with chemotherapeutic agents, and transfection of p53 could not enhance the sensitivity of SMMC-7721 to TRAIL-inducing apoptosis. The results demonstrated TRAIL could decrease the threshold of some chemotherapeutic drugs 50-100 folds.

Low dose of interleukin-2 enhanced TRAIL-inducing apoptosis in HCC cells

HCC cells were cultured in the presence of TRAIL or TRAIL plus IL-2 or IFN-γ. Cultures were examined by phase contrast light microscopy at 6, 12, 24, 48, and 72 h. A rounded cytopathic effect was detectable in HCC cell cultures in the presence of TRAIL plus low dose IL-2 after 12 h, but was more prominent after 24 h. Extensive cell death was apparent in HCC cell cultures treated with TRAIL plus IL-2 at 48-72 h. To quantitate the extent of cell death, we measured cell viability treated with IL-2, TRAIL, or IL-2 and TRAIL using MTT. In contrast, HCC cultures treated with IL-2 alone appeared healthy throughout the experiment. Importantly, the death-inducing synergistic effects of TRAIL in combination with IL-2 were not specific to HCC cells, but also induced other tumor cells death (such as cholangiocarcinoma cell line QBC939, data not shown). These data indicated that TRAIL in combination with low dose IL-2 could induce cell death in HCC cells cultures in vitro. To our surprise, Low dose of IL-2 significantly enhanced TRAIL-inducing apoptosis in HCC cells after 48-72 h, but high dose of IL-2 only had a weaker role.

To delineate that TRAIL and IL-2 specifically killed HCC cells and spared normal liver cells, we cultured primary normal liver cells and normal fetal liver cell line L02 in the presence of TRAIL and IL-2 for 48 or 72 h. We subsequently determined whether they were undergoing apoptosis. By contrast to HCC cells, normal cells cultured in the presence of TRAIL and IL-2 exhibited no signs of apoptosis. These data indicated that TRAIL in combination with IL-2 could selectively induce apoptosis of HCC cells but not normal liver cells. Furthermore, we obtained similar synergistic results with combination of TRAIL and IFN-γ.

Apopotosis detection by FACScan

Cell death might occur through apoptosis or necrosis. Here, we found that TRAIL could kill HCC cells through apoptosis by FACScan (Figure 6).

DISCUSSION

To selectively kill target tumor cells and spare normal tissues are one of the ultimate goals of cancer research. TRAIL, a type II transmembrane protein, was initially identified based on the homology of its extracellular domain with FasL, TNF and lymphotoxin-α[8]. TRAIL transcripts have been detected in the adult spleen, thymus, lung, prostate, ovary, small intestine, peripheral blood lymphocytes, colon, heart, placenta, skeletal muscle, and kidney, but not in the adult liver, brain, and testis. Receptors that bind to TRAIL include death receptor (DR) 4/TRAILR1[10], DR5/KILLER/TRAILR2/ TRICK2[11-16], decoy receptor (DcR) 1/TRAILR3/TRID (TRAIL without an extracellular domain)/LIT (Lymphocyte Inhibitor of TRAIL)[12-15,19], DcR2/TRAILR4/TRUDD (TRAIL receptor
with a truncated death domain)[19-21] and a soluble receptor called osteoprotegerin (OPG)[22] DR4, DR5, DcR1, and DcR2 have been shown to bind TRAIL with similar affinities. However, only two of the TRAIL receptors, DR4 and DR5, contain functional death domains and are capable of inducing apoptosis. DcR1, DcR2 are highly expressed in normal tissues, but have substantially lower expression in malignant cells, indicating a low cytotoxic effect in normal tissues. In contrast, DR4 and DR5 are expressed in malignant cells as well as in normal tissues. Despite having multiple receptors, TRAIL can selectively target tumor cells to undergo apoptosis while leaving normal cells unaffected. The selectivity of TRAIL-induced apoptosis has been demonstrated by systemic administration of TRAIL, which reduces tumor growth in vivo, without the severe toxic effects often seen in TNF or FasL treated mice. A relatively large number of tumor cell lines, such as leukemia, lymphoma, myelanoma, melanoma, breast cancer, colon carcinoma, cholangiocarcinoma or thyroid carcinoma, are sensitive to cytotoxic effects of TRAIL, indicating that TRAIL may be a powerful selective cancer therapeutic[33-35].

However, the potential utility and safety of systemic administration of TRAIL have been recently questioned because results showed sensitivity of human but not monkey or mouse hepatocytes to a polhyside-coded recombinant soluble form of TRAIL (amino acids 114-281, 114-281TRAIL, his)[22]. Fortunately, another study showed that the toxicity of histidine-tagged TRAIL to human hepatocytes in vitro had aberrant biochemical properties, i.e., loss of zinc with the formation of intersubunit disulfide bonds. When this reagent was replaced by a nontagged soluble TRAIL zinc-replete TRAIL (114-281,TRAIL,rs), normal liver cell cytotoxicity was not observed[33-35].

Another potential drawback of TRAIL therapy was that not all cancer cells were sensitive to TRAIL. The regulation of TRAIL signaling is extremely complex, the mechanisms of TRAIL-resistance remain unclear. It has been suggested that the reason why some cells are susceptible to TRAIL while others appear to be resistant lies in the endogenous presence of the non-death-signaling TRAIL[35]. However, examination of various tumor cell types has shown that basal expression of TRAILR did not correlate with susceptibility to TRAIL[36,37]. Alternatively, the presence or absence of intracellular inhibitors of apoptosis such as the cellular inhibitor of caspase 8/Flice-inhibitory protein (cFLIP) might be important[38-44], and in some circumstances, cFLIP expression did correlate with the susceptibility of tumor cells to TRAIL[37]. It has been found that NF-κB activation also protected cells against TRAIL-induced apoptosis[19]. Taken together, previous studies indicated that TRAIL resistance to apoptosis might be cell-type specific requiring up-regulation or down-regulation of one of the TRAIL receptors, assembly of different receptors, involvement of decay receptors, and functions of intracellular inhibitors or intracellular anti-apoptotic/survival pathways working in concert. There is long way to clarify TRAIL-resistance mechanisms. How to reverse TRAIL-resistance is also an important topic before TRAIL is developed into an effective anticancer drug. Therefore, as the first step we detected the assembly of different receptors in HCC.

In the present study, both DR were present in all HCC tissue as well as in normal hepatic tissues derived from surgically resected margins of benign tumor. In contrast, 54 HCC tumors did not express DcR1 and 25 HCC tumors did not express DcR2. Both DcR were detectable in all of the normal liver tissues. High expression of DR and low or no expression of DcR in HCC tissue differed from low expression of DR and high expression of DcR in normal hepatic tissues. DR5, DR4, and DcR2 were expressed in both cell lines examined, but DcR1 did not express in both cell lines. Although expressed detectable TRAIL-R1 and -R2, both HCC cell lines showed strong resistance to TRAIL-induced apoptosis. Recombinant TRAIL (100 ng/ml) alone was found to have a slight activity as it killed about 10% of HCC cells within 24 h compared with over 70% of Jurkat cells and about 50% of human cholangiocarcinoma cell line QBC939. Transfection of TRAIL cDNA also failed to induce extensive apoptosis in HCC lines. In vivo administration of TRAIL gene could not inhibit tumor growth in nude mice HCC model. These results suggest that some possibly endogenous suppressors of TRAIL-mediated apoptosis might exist in HCC cells. Certainly, components for TRAIL-induced apoptosis existed in these cell lines, and resistance to apoptosis in HCC was more likely mediated by intracellular signaling events than by alterations in receptor expression or the presence of decoy receptors. The mechanisms of TRAIL resistance in HCC should be identified in order to reverse TRAIL resistance.

The pharmacokinetic profile of soluble TRAIL indicated that after iv injection the majority of protein was cleared within 5 hours. Increasing in vivo t1/2 of soluble TRAIL or developing an alternative means of delivery may increase the relative tumoricidal activity of TRAIL. Identification of alternate methods to deliver TRAIL to the tumor site is also critical for further developing and testing anti-tumor activity of TRAIL in vivo. The results in our work described the production of an expression vector engineered to carry the gene for TRAIL. Shortly after cell tranfection, TRAIL protein was detected, rapidly leading to the induction of apoptosis in TRAIL-sensitive tumor cells (such as cholangiocarcinoma cell line QBC939, data not shown) in vitro, indicating the potential of using TRAIL gene and local expression of TRAIL to destroy TRAIL-sensitive tumor cells in vivo. As for TRAIL resistant tumor cells, effective treatment would be attached by new therapy strategies such as combining gene therapy.

How to reverse TRAIL-resistance is an important topic before TRAIL is developed into an effective anticancer drug. Consistent with several previous reports, our results showed that chemotherapeutic agents significantly sensitized HCC cell lines to TRAIL-induced apoptosis. TRAIL could decrease the threshold of some chemotherapeutic drugs 50-100 fols. The mechanism of overcoming tumor resistance to TRAIL-induced apoptosis by chemotherapeutic agents must be reserved for further investigation. Therefore, our results also showed that cytokines that compose part of the arsenal of the immune system against tumors might alter HCC sensitivities to TRAIL. For example, a subtoxic level of interleukin-2 could overcome the resistance of HCC cells. No significant apoptosis was induced in cells pretreated with IL-2 alone even at 10 000 unit/ml, and TRAIL (50 ng/ml) induced about 10% cell death after 24 h treatment. However, HCC cells treated with IL-2 (50 unit/ml) in combination with TRAIL (50 ng/ml) were killed about 50% at 48 h, indicating that IL-2 could sensitize HCC cells to TRAIL-induced apoptosis. The effect of IL-2 which enhanced TRAIL-mediated apoptosis was not dose-dependent, low dose IL-2 had a stronger sensitizing effect while high dose IL-2 had a weaker role. HCC cells could become sensitive to TRAIL-mediated cell death after treatment IFN-γ, too. The possible explanations for these effects are that IFN-γ could induce TRAIL expression, up-regulate DR and Caspase 8, significantly decrease NF-κB-activation, and prevent IAP2[45-48]. These findings have therapeutic implications because resistance to apoptosis in HCC cells may be dramatically overcome by agents used in combination.

In summary, TRAILR expression is prevalent in HCC and there is a different expression of receptor types in HCC cells compared with normal hepatic tissue. HCC cells are insensitive
to TRAIL-mediated apoptosis, suggesting the presence of mediators can inhibit the TRAIL-inducing apoptosis pathway in HCC and has a limited therapeutic role for TRAIL as a single agent in HCC. However, TRAIL-based tumor therapy in combination with chemotherapeutic agents or anticancer cytokines might be a powerful treatment for HCC.

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