Tumor Necrosis Factor Related Apoptosis Inducing Ligand (TRAIL)-Mediated Apoptosis in Human Hepatocellular Carcinoma Cells

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Abstract

TRAIL, a novel member of TNF superfamily, induces apoptosis in transformed cell lines of diverse origin. In this study, we investigated TRAIL-mediated apoptosis in human hepatocellular carcinoma (HCC) cells and role of TRAIL receptors in conferring sensitivity to TRAIL induced apoptosis in HCC cells by examining a large panel of human HCC cell lines for their sensitivity to TRAIL-mediated apoptosis. Our results revealed that most of HCC cell lines expressed TRAIL death receptor TRAIL-R1 and TRAIL-R2, but did not express decoy receptorTRAIL-R3. Expression of TRAIL receptors in HCC cells did not correlate with their sensitivity to TRAIL mediated killing. Caspase-3 but not caspase-1 activity is closely related to the sensitivity to TRAIL-mediated apoptosis. Treatment with actinomycin D sensitizes the TRAIL- resistant HCC cell lines to be susceptible to TRAIL-mediated apoptosis, with induced activation of caspase-3. Our results indicated that there are differential regulatory mechanisms controlling TRAIL sensitivity in HCC cells and one is correlated with caspase-3 activation but not with the expression of decoy receptors. (J Intern Med Taiwan 2003; 14: 271- 281)

Key Words：TRAIL death receptors; Decoy receptors; Apoptosis; Caspase-3

Introduction

TRAIL, a novel member of TNF superfamily,TRAIL have been shown to be able to induce rapid apoptosis in a variety of transformed cell lines 1,2. It was known recently that TRAIL displays potent anti-tumor activity against selected targets and monocyte-mediated tumor cell apoptosis was TRAIL specific 3. This implies that TRAIL may be a key effector molecule in anti-tumor activity in vivo. Four receptors

for TRAIL have been identified. The ability to transduce death signals is restricted to DR4/TRAIL-R1 and DR5/TRAIL-R2 (hereafter referred to as TRAIL-R1 and TRAIL-R2, respectively) 4-7. In contrast, TRID/DcR1/TRAIL-R3 and DcR2/TRAIL-R4 (hereafter referred to as TRAIL-R3 and TRAIL-R4, respectively) lack functional death domains and are unable to activate apoptosis 5,6,8-10. Furthermore, it has been shown that these two inhibitory receptors could inhibit TRAIL-mediated apoptosis, and TRAIL-R3 and TRAIL-R4 were suggested to act as "decoy receptors" to protect normal tissues from cell death 8-10. Based on the selective expression of TRAIL-R3 on normal tissue but not in transformed cell lines, suggesting that TRAIL may be involved in tumor killing in vivo 5,6. In fact, it has been demonstrated that systemic administration of TRAIL could suppress tumor growth in vivo without affecting normal tissues in mice 11.

Human HCC is a highly malignant cancer and shows strong resistance against current therapy. Therefore, we intended to investigate TRAIL induced killing in human HCC cells to test the potential role of TRAIL-mediated apoptosis in treatment of human HCC. In this study, we investigated TRAIL-mediated apoptosis in human HCC cells and role of TRAIL receptors in conferring sensitivity to TRAIL induced apoptosis in HCC cells by examining a large panel of human HCC cell lines for their sensitivity to TRAIL-mediated apoptosis. Here, we demonstrated that human HCC cells showed variable sensitivity to TRAIL-mediated apoptosis and there are differential regulatory mechanisms controlling TRAIL sensitivity. Our results provide information concerning the regulation of TRAIL-induced apoptosis in human HCC cells, and suggest a potential use for TRAIL as a treatment for HCC.

Materials and Methods

Cell lines

The Jukat T cell lines, the human HCC cell lines, HepG2 12, Hep3B Hep3B 13, and SK-Hep1 cells were purchased from American Type Culture Collection (Rockville, MD). The human HCC cell lines: Tong, Huh7 Huh7 12, HA22T, NTU-BL, PLC5 14-15, Mahlavu 16, 59T, ChangLiver, HCC36 were obtained from Dr. Lih-Hwa Hwang (Hepatitis Research Center, National Taiwan University Hospital).

All the human HCC cell lines as well as human cervical carcinoma cell line, HeLa cell were grown in DMEM supplemented with 10% FBS, 100U/ml penicillin, 100 mg/ml streptomycin, and nonessential amino acids. The human T cell leukemia line, Jurkat cell was grown in RPMI 1640 supplemented with 10% FBS, 100U/ml penicillin, and 100 mg/ml streptomycin. All the cells were incubated at 37℃ in a 5% CO2 incubator.

RT-PCR of TRAIL and TRAIL receptors in HCC cell lines

Total RNA was isolated from a fixed number of cells (5 x 106) in 1 ml of TRizol

reagent (Gibco BRL, Grand Island, NY, USA) and 100µl of chloroform following the manufacturer's instructions. The RNA pellets were dried and resuspended in 50µl of diethylpyrocarbonate-treated double distilled water. The concentrations of RNAs were measured by spectrophotometer. The cDNA was generated from 2.5µg of extracted RNA using Superscript IITM (50U/µl, Gibco BRL, Grand Island, NY, USA) and 2.5µl random hexamer (50 ng/µl) in a final volume of 20µl. The TRAIL specific primers were: sense, 5'-GCGGGTACCGAATTCAGAACCTCTGAGGAA-ACC-3'; and antisense, 5'-GCGG GTACCCAGGTCAGTTAGCCAACT-3'. The TRAIL-R1 specific primers were: sense, 5'-GCTGAGCAACGC-AGACTCGCTGTCCAC-3'; and antisense, 5'-GTCCAAGG-ACACGGCAGA-GCCTGTGCCAT-3'. The TRAIL-R2 specific primers were: sense, 5'-GGCCTCATGGACAATGAGATAAAGGTGGCT-3'; antisense, 5'-GCCAAATCT-CAAAGTACGCACAAACGG-3'. The DcR1/TRAIL-R3 specific

primers were: sense, 5'-GGAAGAATTTGGTGCCAATGCCACTG-3’ antisense,

5'-GCTCTTGGACTT-GGCTGGGAGATGTG-3'. The sequence of DcR2/TRAIL-R4 specific primers were: sense, 5'-GCTTTTCCGGCGGCGTTCATGTCCTTC-3' ; antisense, 5'-GGTTTCTTC-CAGGCTGCTTCCCTTTGTAG-3'. The Fas and FasL specific primers were: sense, 5'-GGAAGGATCCAGATCTAACTT-3'; antisense,

5'-GGTACAAAGATTGGCTTTTTT-GAGA-3’

and sense, 5'-GGGAATTCCAGCTGCCATGCA-GCAGCCC-3'; antisense, 5'-GGGAATTCCCTCTTAGAGC-TTATATAAGCCG-3', respectively. The _-actin specific primers were: sense, 5'-GGTGGGGCGCC-CCAGGCACCA-3'; antisense, 5'-GCTCCTTAATGTCACGCACGATT TC-3'.

The PCR reactions were performed in a 50 μl total reaction volume using the DNA thermocycler 480 (Perkin Elmer, Norwalk, CT, USA) and Tag DNA polymerase (5U/ μl, Promega, Madison, WC). cDNA was first kept at 95℃ for 5 minutes, and was subject to amplification reaction of 1 minute at 95 ℃, 1 minute at 55℃, and 1 minute at 72℃ per cycle for 30 cycles and then 10 minutes at 72℃. A 20 μl of each RT-PCR reaction product was electrophoresed on a 2% agarose gel in TAE buffer. The products (625 bp for TRAIL, 506 bp for TRAIL-R1, 502 bp for TRAIL-R2, 612 bp for TRAIL-R3, 453 bp for TRAIL-R4, 413 bp for Fas, 871 bp for FasL, 550 bp for β-actin) were visualized under UV light after ethidium bromide staining.

Ribonuclease Protection Assay (RPA)

The RPA assay was performed with hAPO-3c multi-probe template set and the RiboQuant multi-probe ribonuclease protection assay system according to the manufacturer's recommendations (PharMingen, SanDiego, CA, USA).

High-specific-activity RNA probes of caspase 8, Fas, FasL, TRAIL, TRAIL-R1, TRAIL-R2, TRAIL-R3, TRAIL-R4, TNFRp55 (TNF receptor p55), TRADD (TNF receptor-associated death domain protein), RIP (receptor-interacting protein), L32 (ribosomal protein), and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) were synthesized. In brief, a mixture of 1 μl RNasin (32 U), 1 μl GACU pool (0.5mM), 2 μl DTT (10 mM), 4 μl 5X transcription buffer, 10 μl [α-32P] UTP (120 μCi), 1 μl RNA polymerase, and 1 μl RPA template set were incubated at 37℃ for 1 hour. A 2 μl DNase was added into the reaction and the reaction was incubated at 37℃ for 30 minutes. The yeast tRNA and 26 μl 20mM EDTA was added and the reaction was subject to phenol/chloroform and chloroform extraction. The RNA probes were precipitated with 1 volume of 4 M ammonium acetate and 5 volume of ethanol for 30 minutes at -70℃. The RNA pellet was washed once with 90% ethanol, air-dried and resuspended in 50 μl hybridization buffer.

Total RNAs of human HCC cell lines were extracted using TriZol reagent (GibcoBRL) according to the manufacturer's suggestions. The RNAs were stored in RNAse-free water in -70℃. A 2 μg of RNA was resuspended into hybridization buffer and proper amount of diluted probes was added. The tubes were briefly heated at 90℃ and incubated at 56℃ for 12-16 hours. The tubes were then incubated at 37℃ for 15 minutes followed by RNase A and T1 digestion at 30℃ for 45 minutes. The reactions were then subject to proteinase K digestion. The yeast tRNA was added into the reaction, and the tubes were subject to phenol/chloroform and chloroform extraction. The reactions were then treated with 1 volume of 4M ammonium acetate and 5 volume of 100% ethanol at -70℃ for 30 minutes. The RNAs were then washed with 90% ethanol, air-dried and resuspended into 5 μl of loading buffer. The samples were heated at 90℃ for 3 minutes and were subject to acrylamide gel electrophoresis. The gel was dried under vacuum for 1 hour at 80℃. The radioactivity was shown on Phosphoimaging (Molecular Dynamic, Sunnyvale, CA) and X-ray densitography using Kodak X-AR films.

Apoptosis assay

Apoptosis of HCC cell lines was measured by crystal violet staining method 17. In brief, cultured cells were treated with trypsin-EDTA, counted, and 104 cells were suspended in complete DMEM supplemented with nonessential amino acids, plated in 96-well culture plates (Corning Science Products. Acton, MA, USA) and cultured overnight at 37℃ in a 5% CO2 incubator. Medium was removed the next day, and 100 μl of fresh medium with 10% FCS and recombinant TRAIL protein (1400 ng/ml to 87.5 ng/ml) was added. Cells were incubated for further 24 hours at 37℃ and washed three times with PBS. Cells were incubated with 200 μl of 4% paraformaldehyde for 10 minutes at room temperature, washed three times with PBS,

and incubated with 50 μl crystal violet (1%) for 20 minutes at room temperature. Cells were then washed three times with PBS, dried, and a 200μl crystal violet lysis buffer (33% acetic acid in ddH2O) was added. The absorption at OD550 was detected by spectrophotometer. Specific killing was calculated as follows: specific killing (%) = 100 x (OD550 of control cells - OD550 of experimental cells) / OD550 of control cells. For some condition, human HCC cells lines were cultured with recombinant TRAIL in the presence of actinomycin D (0.05 μg/ml) for 24 hrs, then were subject to TRAIL-mediated apoptosis assay by crystal violet.

Caspase Activity Assay

Cell were seeded onto 6-well plates, and pretreated with 1% serum for 24 hr. Then cell were incubated with or without TRAIL (1.5μg/ml) for 24 hr. Cells were lyzed in TENN buffer containing 50mM Tris-HCl (PH7.4). 5 mM EDTA, 0.5% Nonidet p-40, 150 mM NaCl, 1 mM phenylmethylsufonyl fluride, 2μg/ml leupetin, and 2μg/ml pepstatin and cell lysate was centrifuged at 14,000 rpm. The supernatant was assayed for protein concentration. Cell lysate with 100μg proteins in 1 ml assay buffer containing 50 mM Tris-HCl (PH7.4), 1 mM EDTA and 1 mM EGTA was incubated with 5 μM fluorogenic peptide substrates (Ac-DEVD-AMC for caspase-3 and Ac-YVAD-AMC for caspase-1) at 37℃ for 30 min. The fluorescence generated was quantified in a spectrofluorometer (Wallac/PerkinElmer Inc. Boston, MA) (excitation 460 nm, emission 355 nm).

Results

TRAIL induced cell death in human HCC cell lines

TRAIL was shown to induce apoptosis in a number of different tumor cell types, but usually not in normal primary cells. To examine a role for TRAIL-induced apoptosis in human HCC, recombinant TRAIL proteins were used to induce apoptosis in human HCC cell lines. The recombinant TRAIL proteins were expressed in E. coli system and purified with Ni-NTA resin column. The purified recombinant soluble TRAIL protein induced apoptosis in TRAIL-sensitive target, Jurkat cell in vitro 18. We then used this recombinant TRAIL protein to analyze the sensitivity of human HCC cell lines to TRAIL induced cell death.

The susceptibility of human HCC cells to TRAIL-mediated apoptosis was studied over a range of concentrations of recombinant TRAIL proteins. As shown in Figure 1, the human HCC cell lines demonstrated variable sensitivity to TRAIL-mediated apoptosis. Among 10 human HCC cell lines analyzed, the HCC cell lines showed sensitive, partial or complete resistance to TRAIL-mediated apoptosis in various degrees (Figure 1). Three cell lines (Mahlavu, HA22T and ChangLiver) showed strong sensitivity to TRAIL induced cell death. Cellular apoptosis reached up to 60% at 750 ng/ml of recombinant TRAIL protein. Other HCC cell lines showed less

than 20% of cell death after incubation with 750 ng/ml of recombinant TRAIL protein (Figure 1).

Expression of TRAIL and TRAIL receptors in human HCC cell lines

We then investigated the expression of TRAIL and TRAIL receptors by RT-PCR in 12 human HCC cell lines (Huh7, Tong, Hep3B, HA22T, HepG2, NTU-BL, PLC5, SK-Hep1, Mahlavu, 59T, Changliver and HCC36) as well as human T leukemia cell line, Jurkat, human cervical carcinoma cell line, HeLa and human PBMC as control. Amplification of human β-actin served as a control for sample loading and integrity. The results of RT-PCR shown in Figure 2 demonstrated that normal human PBMC expressed TRAIL and all the four TRAIL receptors. Among human HCC cell lines, TRAIL, TRAIL-R1 and TRAIL-R2 were expressed in most of the cell lines analyzed. But TRAIL-R3 was not expressed in any of these cell lines. In contrast, TRAIL-R4 was detected in all 12 HCC cell lines and HeLa cell. This result is consistent with previous observation in other transformed cell lines, which suggests that TRAIL-R3 is selectively expressed in normal cells, but not in transformed cell lines.

For further quantitative analysis of TRAIL receptor expression in human HCC cell lines, we used RPA to quantify the expression of TRAIL receptors as well as Fas L, TRAIL, TNFRp55, caspase 8, TRADD and RIP in four HCC cell lines (HA22T, HepG2, Mahlavu and SK-Hep-1) and HeLa cell

(Figure 3). The results demonstrated that there was weak TRAIL expression in most of the cell lines analyzed but the expression pattern of TRAIL receptors and caspase-8 as well as other death molecules was similar among HCC cell lines and HeLa cell. The expression level of TRAIL-R1, R2, and R3 in each HCC cell line was further analyzed in densitometry and plotted in Figure 3B. The results indicated that among the TRAIL receptors, TRAIL-R2 showed the strongest signal in all the HCC cell lines analyzed. This suggested that TRAIL-R2 is the predominant TRAIL receptor expressed in HCC cell lines. TRAIL-R1 expressed in all the HCC cell lines but with 25-50% expression level of TRAIL-R2. There was weak expression of TRAIL-R3 in SK-Hep1 and Mahlavu (12-20% of TRAIL-R2) but no TRAIL-R3 expression in HepG2 and HA22T.

Resistant HCC cell lines became sensitive to TRAIL-mediated apoptosis in the presence of actinomycin D

Several of the human HCC cell lines were resistant to TRAIL-mediated killing. We further studied TRAIL-induced apoptosis in three of the resistant cell lines Huh7, PLC5 and HepG2, under different conditions. In the presence of a sub-toxic level (0.05 μg/ml) of actinomycin D, these cell lines became sensitive to TRAIL-induced cell death (Figure 4). The TRAIL induced cell death could reach from below 20% up to 54% (Huh7), 64% (PLC5) and 88% (HepG2) in the presence of actinomycin D

(Figure 4). Similar results were obtained in other HCC cell lines. In addition to resistant HCC cell lines, there is also enhanced TRAIL sensitivity observed in TRAIL-sensitive HCC cell lines in the presence of sub-toxic level of actinomycin D (data not shown).

TRAIL-induced caspase activation in HCC cell lines

To determine at what level of the TRAIL-induced apoptosis signaling transduction pathway might be regulated in HCC cell lines, we investigated the activation of caspases in HCC cells induced by TRAIL. The HCC cell lines HepG2 and SK-Hep1 were resistant to TRAIL induced cell death and had minimal activation of caspase 3 and caspase 1 after induction by TRAIL. The results in Figure 5 demonstrated that treatment of actinomycin D converted the TRAIL-resistant HCC cell lines to be TRAIL-sensitive, and also upregulated the caspase-3 activity in these cell lines. Similar results were obtained in other HCC cell lines (data not shown). This indicated that caspase-3 activation is critically involved in TRAIL death signal transduction in human HCC cell lines. It also suggested that the regulation of TRAIL sensitivity is controlled at the upstream of caspase-3. In contrast, caspase-1 activation was not involved in regulation of TRAIL-induced apoptosis signaling pathway in human HCC cell lines.

Discussion

In the present study, we examined a large panel of human HCC cell lines for their susceptibility to TRAIL and demonstrated that there is variable sensitivity to TRAIL-mediated apoptosis in human HCC cell lines and some of them showed high sensitivity to TRAIL-mediated apoptosis in vitro. We further demonstrated that the TRAIL-sensitive and -resistant HCC cell lines showed a similar pattern of expression of TRAIL receptors. It indicated that there was differential regulation of sensitivity to TRAIL induced cell death among HCC cell lines besides expression of TRAIL receptors. Among the TRAIL receptors, TRAIL-R2 showed the strongest signals among all HCC cell lines in RPA, suggesting that TRAIL-R2 may be the predominant TRAIL receptors expressed in HCC cell lines. It is possible that TRAIL-R2 is also the major TRAIL receptor expressed in vivo. The HCC cell lines expressed most of the TRAIL receptors including TRAIL-R1, TRAIL-R2 and TRAIL-R4, except TRAIL-R3. This observation is consistent with previous observations in other transformed tumor cell lines, which suggested that TRAIL-R3 served as a role of decoy receptor 8-10. However, in our results, we demonstrated that the sensitivity to TRAIL-mediated apoptosis in HCC cell lines was not contributed to the expression of specific TRAIL receptors. This is consistent with the results in recent studies in human malignant glioma and neuroblastoma cell lines 19-20. This indicated the complex interplay of TRAIL receptor signaling in controlling the sensitivity to TRAIL induced cell death.

In our results, we demonstrated that HCC cell lines expressed TRAIL-R4 but not TRAIL-R3. Both TRAIL-R3 and TRAIL-R4 have been suggested to serve as "decoy receptor" and could provide protection against TRAIL-mediated apoptosis in vitro 4,8-10,21. TRAIL-R4 was expressed in both TRAIL sensitive and resistant HCC cell lines. It implied that TRAIL-R4 might not play a critical role in regulation of susceptibility to TRAIL-mediated apoptosis in HCC cell lines. Therefore, our results did not support a role of "decoy receptor" for TRAIL-R4 in HCC cells. Another "decoy receptor" TRAIL-R3 is complete without intracellular domain and was shown to block TRAIL-mediated apoptosis in lymphocytes 22. It has been demonstrated that TRAIL-R3 was expressed in normal cells, whereas its expression was relatively low in cancer cell lines 4,6,23. Similarly, we detected very low or no expression of TRAIL-R3 in the HCC cell lines. It is still not clear the actual biological function of the TRAIL-R3 in vivo. In a recent report, Yamanaka et al. also demonstrated that TRAIL-R1 and -R2 were expressed on human HCC tissues 24, suggesting that HCC cells could be sensitive to TRAIL. However, they didn't study the expression of TRAL-R3 on primary HCC cells and it is not known whether primary HCC cells express TRAIL-R3 or not. It has been demonstrated that post-translational control mechanism was related to the expression of TRAIL receptors in melanoma cell lines 25. Further immuno-histochemistry studies to distinguish the surface expression of TRAIL-R3 between normal hepatocytes and primary HCC cells will be helpful to answer this question. Moreover, from the results that expression pattern of TRAIL receptors is similar between TRAIL-resistant and -sensitive HCC cell lines, our data do not support the notion that expression of TRAIL receptor plays important role in regulation of sensitivity to TRAIL in HCC cells. In addition to the four TRAIL receptors, another soluble receptor of TNF receptor family, OPG, has been shown to bind to TRANCE and TRAIL and has been considered as the fifth TRAIL receptor 26-27. It would be interesting to know whether OPG play a role in regulation of TRAIL mediated killing. However, this possibility seems not likely because OPG existed as a soluble receptor instead of expression on the cell surface. Furthermore, OPG is with much lower affinity to bind to TRAIL compared to other TRAIL receptors 28. Therefore, it is not likely that OPG plays a major role in regulation TRAIL sensitivity in HCC cells.

In the present study, we demonstrated that TRAIL-resistant HCC cells could be converted to TRAIL-mediated killing in the presence of actinomycin D (Figure 4). The results indicated that some short life intracellular regulators might regulate TRAIL-transduced death signal in human HCC cells. Our results showed that caspase-3 but not caspase-1 activity is closely related to the sensitivity to TRAIL-mediated apoptosis in HCC cell lines. Treatment of actinomycin D in

TRAIL-resistant HCC cell lines, rendered them become sensitive to TRAIL-induced cell death, and also upregulated their caspase-3 activity (Figure 5). The demonstration of up-regulation of caspase-3 activity in these cells indicated that regulation of TRAIL death signal transduction in these cells may occur at upstream of Caspase-3. Caspase-1 was not involved in the regulation of TRAIL death signaling pathway in HCC cells. Among the pathways upstream caspase-3, the mitochonrial pathway has been demonstrated to be play an important role in regulating TRAIL-mediated apoptosis 29-30. Recently there are several reports using tumor cell lines with Bax deletion 29-30 or selected for Bax mutation 29 showed that Bax was required for TRAIL-mediated apoptosis 29. Thus, in these cells, the mitochondrial pathway was required for TRAIL-mediated apoptosis, and Bax was essential for the mitochondrial events.

Our studies indicated the potential efficacy of TRAIL-mediated apoptosis in the treatment of HCC. In a recent report demonstrated that chemotherpeutic agents augment TRAIL induced apoptosis in human HCC cell lines 24, and this would imply that TRAIL-based tumor therapy in combination with chemotherpeutic agents might be a powerful potential therapeutic tool in treatment of human HCC. However, recently Jo et al. demonstrated that TRAIL could induce apoptosis in human primary hepatocytes but not hepatocytes in other species in vitro 31. The results demonstrated the different susceptibility of hepatocytes to TRAIL-mediated apoptosis in humans and other species. Therefore, the potential use of TRAIL in treatment of human HCC still awaits further studies. The finding that HCC cells can be sensitive, partially or complete resistant to TRAIL suggests the presence of more complex regulatory mechanisms in TRAIL-induced apoptosis. Understanding the control mechanisms in regulation of TRAIL induced apoptosis will help us to optimize TRAIL and other combinatorial therapy for treatment of human HCC.

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Fig.1. Recombinant TRAIL protein-mediated apoptosis in human HCC cell lines. 1 x 104 cultured human HCC cell lines were plated in 96 wells overnight, and were incubated with different concentration of TRAIL fusion protein as indicated in the figure and cultured for another 24 hours. Cell death was assayed by crystal violet staining. The HCC cell lines Mahlavu, HAT22 and Changliver are sensitive to TRAIL-mediated apoptosis, while most of the other HCC cell lines are resistant to TRAIL-mediated apoptosis.

Fig.2. Expression of TRAIL and TRAIL receptors on human HCC cells. Expression of TRAIL, TRAIL-R1 to TRAIL-R4, Fas and FasL in human PBMC, human HCC cell lines, Jurkat cell and HeLa cell lines by RT-PCR assays. Total RNA was isolated from human HCC cell lines, human PBMC, Jurkat and HeLa cells. The cDNAs was reversed transcribed and PCR reactions were performed with equal amounts of cDNA. Amplification with human β-actin primers served as a control for equal loading and integrity. The amplified DNA was separated by agarose gel electrophoresis, and visualized by ethidium bromide staining.

Fig.3. Quantitative expression of TRAIL, TRAIL receptors in human HCC cell lines and HeLa cell by RNase protection assay (RPA). A. Detection of TRAIL, TRAIL receptors, FasL and other death molecules in human HCC cell lines. The HCC cell lines HepG2 and SK-Hep1 were resistant to, while Mahlavu, HAT22 and human cervical cancer cell lines HeLa were sensitive to TRAIL-mediated apoptosis. B. Quantitative analysis of TRAIL receptor expression level in human HCC cell lines. The image intensity of TRAIL-R1 to -R3 and L32 in RPA was quantitatively analyzed in densitometry.

Fig.4. Human HCC cell lines resistant to TRAIL-mediated apoptosis were converted to sensitive to TRAIL in the presence of actinomycin D. HCC cells lines were cultured with recombinant TRAIL (1.5μg/ml) in the presence of actinomycin D (50 ng/ml) for 24 hrs, and were tested for the sensitivity to TRAIL-mediated apoptosis. The cell death was analyzed by crystal violet assay.

Fig.5. Caspase activation after TRAIL-engagement in HCC cell lines.
TRAIL-resistant cell lines (HepG2 and SK-Hep1) were incubated with recombinant TRAIL for 24 hr in the presence of or absence of actinomycin D. The cells were then lysed and the supernantant was further incubated with fluorogenic peptide substrates

for caspases (Ac-DEVD-AMC for Caspase 3 and Ac-YVAD-AMC for Caspase 1) at 37℃ for 30 min. The fluorescence generated was quantified in a spectrofluorometer.

人類肝癌細胞在 TRAIL 所引致細胞凋亡之研究

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摘 要

TRAIL 是腫瘤壞死因子(TNF)家族的一員。在各種不同來源的轉形細胞株引致細胞凋亡作用。我們研究在不同人類肝癌細胞株的 TRAIL 受體表現，以及這些不同人類肝癌細胞株對於 TRAIL 所引致細胞凋亡作用的感受性。結果發現大部份的人類肝癌細胞株表現出 TRAIL 的死亡受體 TRAIL-R1 及 TRAIL-R2；但並不表現誘餌受體 TRAIL-R3。本研究發現人類肝癌細胞株對於 TRAIL 所引致細胞凋亡作用的感受性並非是決定於 TRAIL 受體的表現；引起人類肝癌細胞對於 TRAIL 所引致細胞凋亡作用的感受性與細胞內所引發之 caspase 3 的活性有關，但與 caspase 1 的活性無關。而當先以 actinomycon D 處理肝癌細胞株， 可使肝癌細胞株對於 TRAIL 所引起細胞凋亡作用敏感性增加，同時活化細胞內 caspase 3 的活性。本研究顯示人類肝癌細胞株藉由不同的層級控制對於 TRAIL 引起之細胞凋亡作用，而其中之一的控制機轉與細胞內 caspase 3 之活化相關，而與 TRAIL 受體的表現無關。