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Advances in Brief

Adenoviral Gene Transfer of Tumor Necrosis Factor-related Apoptosis-Inducing Ligand Overcomes an Impaired Response of Hepatoma Cells but Causes Severe Apoptosis in Primary Human Hepatocytes[1]

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Abstract

Ligands of the tumor necrosis factor family play key roles in liver pathogenesis. The ligand tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is unique, because it is thought to be nontoxic to normal cells while killing a broad range of tumor cells. However, hepatocellular carcinoma is considered resistant to soluble TRAIL treatment. Therefore, a direct gene transfer of *TRAIL* to malignant cells is part of an alternative delivery strategy. We show that an adenoviral gene transfer (Ad-*TRAIL*) overcomes an impaired response of hepatocellular carcinoma cell lines to soluble TRAIL, but the transduction of primary human hepatocytes revealed a high number of apoptotic cells. Our data imply that Ad-*TRAIL* administration *in vivo* must either be restricted to tumor tissue or controlled by a tumor-specific promoter to avoid severe liver damage in human trials.

Introduction

Ligands of the TNF[4] family and their cognate receptors play a key role in liver pathogenesis (1). One of these ligands, TRAIL, is unique, because it has been reported to be nontoxic to normal cells while having the potential to selectively kill tumor cells (2, 3). Different disease entities have been evaluated for potential therapeutic effects of TRAIL, including HCC. It was found that despite a strong and prevalent expression of the proapoptotic TRAIL receptors (TRAIL-R1 and -R2) on the surface of HCC cells, none of them showed clear signs of apoptotis after incubation with a sTRAIL preparation (4). Therefore, an alternative way to deliver TRAIL is considered to be a gene therapeutic approach, in which the gene encoding the TRAIL protein is directly transduced into tumor cells by different methods (5–10). However, because the discovery that a certain preparation of sTRAIL can lead to a massive apoptosis of PHHs (11, 12), each and every new application of TRAIL has to be tested for liver toxicity in detail. In particular, for a potential HCC treatment with TRAIL coding vectors, the close contact between hepatoma cells, as a target of the gene transfer, and hepatocytes warrants further investigation, because both cell types seem to share a similar distribution pattern of TRAIL receptors on their surface (4, 11). Here, we show for the first time that an impaired response of human-derived HCC cell lines to sTRAIL can be overcome by an adenoviral *TRAIL* gene transfer, but additional investigations using PHH from different donors clearly demonstrated a strong apoptosis induction and caspase activation in these primary cells. As a broad implication, our data demonstrate that the administration of vector-encoded TRAIL in future human trials must be either strictly limited to tumor tissue or restricted at the transcriptional level to avoid severe liver damage.

Materials and Methods

Virus and Cells. PHHs were kindly provided from the transplantation unit of the University Clinic of Tübingen, R. Deglmann (Munich, Germany), and T. S. Weiss (Regensburg, Germany) and cultured as described previously (13). The human HCC cell lines Huh7, HepG2, and PLC/PRF/5 were obtained from American Type Culture Collection (Rockville, MD) and maintained as recommended by the supplier. Media and supplements were purchased from Life Technologies, Inc. (Karlsruhe, Germany). The adenoviral vector Ad-*GFP* (14) has been described previously, Ad-*TRAIL* was a kind gift of T. Griffith, University of Iowa (7), and Ad-*FasL* was a kind gift of E. Svensson, University of Chicago. Soluble recombinant human TRAIL comprising residues 114–281 was purchased from Calbiochem (Schwalbach, Germany).

Transduction of Cells. Cells were seeded out in 35-mm plates and infected when monolayers had reached 85–90% confluency. As standard inoculation procedure, monolayers were washed twice with medium lacking FCS (washing medium) and overlaid with washing medium containing adenovirus at a MOI of 10 or 100. After incubation for 4 h at 37°C, nonadsorbed virus was removed, and medium containing FCS (growth medium) was added for an incubation over various periods of time (37°C).

Flow Cytometry and Assessment of Caspase Activity. Fragmentation of genomic DNA to hypodiploid DNA was assessed by FACS analysis as described previously (15). For fluorimetric assays of caspase activity, cytosolic extracts of 5×10^4 cells were prepared in lysis buffer [0.5% NP40, 20 mM HEPES, (pH 7.4), 84 mM KCl, 10 mM $MgCl_2$, 0.2 mM EDTA, 0.2 mM EGTA, 1 mM DTT, 5 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin, and 1 mM phenylmethylsulfonyl fluoride]. Caspase-3-like activity was determined by incubation of cell lysates with 50 μM concentration of the substrate *N*-acetyl-Asp-Glu-Val-Asp-aminomethylcoumarin (Ac-DEVD-AMC; Biomol, Hamburg, Germany) in 200 μl of buffer containing 50 mM HEPES (pH 7.3), 100 mM NaCl, 10% sucrose, 0.1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, and 10 mM DTT. The release of aminomethylcoumarin was measured in a kinetic by spectrofluorimetry (excitation wavelength of 360 nm, emission wavelength of 475 nm). Caspase activity was determined as the slope of the resulting linear regressions and expressed in arbitrary fluorescence units per minute.

Immunofluorescence Staining. Microscopy was performed as described elsewhere, using an anti-TRAIL antibody (1 μg/ml MAB375; R&D Systems, Wiesbaden, Germany). Slides were mounted in a Mowiol 40–88 (Sigma, Deisenhofen, Germany) preparation containing 2.5% DA 1,4-Diazabicyclo [2.2.2]octane (DABCO) (Sigma) as an antifade reagent and examined (Olympus fluorescence microscope).

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[4] The abbreviations used are: TNF, tumor necrosis factor; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; sTRAIL, soluble recombinant tumor necrosis factor-related apoptosis-inducing ligand preparation; Ad, adenovirus; GFP, green fluorescence protein; HCC, hepatocellular carcinoma; FACS, fluorescence-activated cell sorter; PHH, primary human hepatocyte; MOI, multiplicity of infection.

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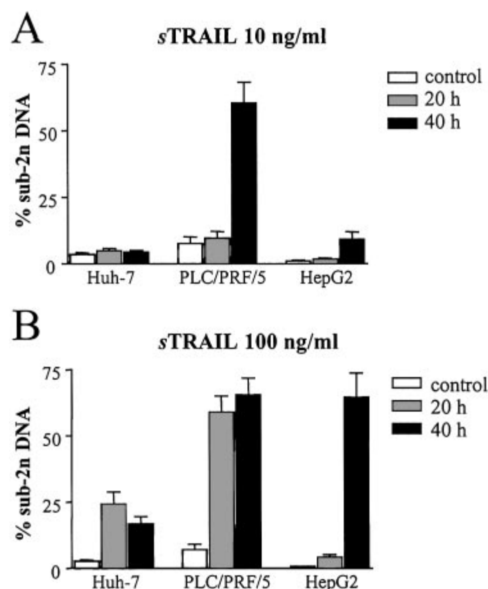

Fig. 1. TRAIL sensitivity of human hepatoma cell lines. Different hepatoma cell lines were incubated with either 10 (*A*) or 100 ng/ml (*B*) sTRAIL protein for 20 or 40 h. The amount of apoptotic cells was determined by FACS analysis (detection of sub-2n DNA). Shown are the results of two independent experiments, including single SD.

ELISA. Maxisorp plates (96 well; Nunc, Karsruhe, Germany) were coated with 1 μg/ml rabbit anti-TRAIL antibody (PeproTech, London, United Kingdom) in 0.05 M carbonate buffer (pH 9.6) overnight at 4°C and stored at -20°C for $\leq$30 days. Plates were washed with 0.05% Triton X-100 in PBS and blocked with 0.5% low-fat milk for 1 h at room temperature. Cell culture media (50 μl), cell lysates (for preparation, see above), and standards from recombinant human TRAIL were adsorbed in triplicates overnight at 4°C. Plates were again washed three times, and adsorbed TRAIL was detected by incubation for 1 h with 2.5 ng/ml mouse anti-TRAIL (R&D Systems) and 0.25 ng/ml peroxidase (POD)-goat antimouse IgG (Bio-Rad, Eggersheim, Germany). Colorimetric reaction with FastRed (Sigma, Munich, Germany) was stopped with 1 N sulfuric acid. Unknown concentrations were calculated from the plot of absorption at 495 nm of known TRAIL standards.

Results

Partial Response of Human Hepatoma Cells to sTRAIL. In a previous study, several human-derived HCC cell lines (Huh7, HepG2, PLC/PRF/5, Hep3B, SK-Hep1, and HLE) were found to be resistant even against incubation with high doses of sTRAIL (100 ng/ml), thereby questioning the usefulness of a systemic administration of sTRAIL as a potential HCC treatment strategy (4). In these experiments, quantification of target cell death was stopped after a short time period of 24 h only. To further extend our knowledge on sTRAIL-specific effects on human HCC cell lines, we now investigated: (*a*) the apoptotic effect in dependence to different sTRAIL concentrations (low dose at 10 ng/ml and high dose at 100 ng/ml) in combination with (*b*) a study of the respective low- and high-dose apoptosis kinetics (0, 20, and 40 h). For this purpose, human HCC-derived cell lines HepG2, Huh7, and PLC/PRF/5 were used.

As a result, incubation with low dosage sTRAIL (10 ng/ml) did not exhibit any specific apoptotic effect on cell lines HepG2 and Huh7 (Fig. 1*A*). However, while at 24 h, PLC/PRF/5 hepatoma cells still exhibited a close to baseline sub-2n level (Fig. 1*A*), a substantial amount of apoptotic cells was detected for PLC/PRF/5 hepatoma cells after a 40-h period of continued incubation (Fig. 1*A*). These results could be confirmed by a corresponding increase of caspase-3 activity under the same incubation conditions, showing another sign of apoptosis induction in these cells (data not shown). Interestingly, under incubation with high dosage sTRAIL (100 ng/ml), HepG2 cells also became highly apoptosis sensitive to the TRAIL treatment after 40-h incubation (71% apoptotic cells), whereas Huh7 cells only showed a moderate increase in the number of apoptotic cells (to 29%) after 20 h of incubation, followed even by a small decline after 40 h of incubation (Fig. 1*B*). These results could be confirmed by an increase of caspase-3 activity.

Taken together, our results demonstrate a partial apoptosis sensitivity of HepG2 and PLC/PRF/5 hepatoma cells under a prolonged period of incubation and the usage of a high sTRAIL dosage. Given the pharmacological profile of sTRAIL with an elimination half-life of $<$5 h after i.v. injection (2, 16), these conditions are not transferable for any *in vivo* treatment strategy of HCC. Stimulated by a recent study that a partial or complete sTRAIL-resistant phenotype exhibited by human prostate cancer cells could be overcome by a direct transfer of the *TRAIL* gene (17), we subsequently explored the potential of an adenoviral *TRAIL* gene transfer to HCC-derived cell lines as a possible new HCC treatment strategy.

Cell-bound Synthesis of TRAIL after Adenoviral Transduction of Liver-derived Cell Types. The use of an adenoviral vector coding for the entire amino acid sequence of TRAIL (Ad-*TRAIL*) has been described recently (7, 8). To ensure that in the context of hepatocytes Ad-*TRAIL* expression leads to a strong and cell-bound gene expression, we first transduced PHHs with two different amounts of Ad-*TRAIL* (MOI of 10 or 100) and looked for intracellular TRAIL synthesis by immunofluorescence microscopy. As a result, we found a strong gene expression in $>$30% of cells using a MOI of 10 and in $>$90% of cells using a MOI of 100 (Fig. 2*A*). The transduction of

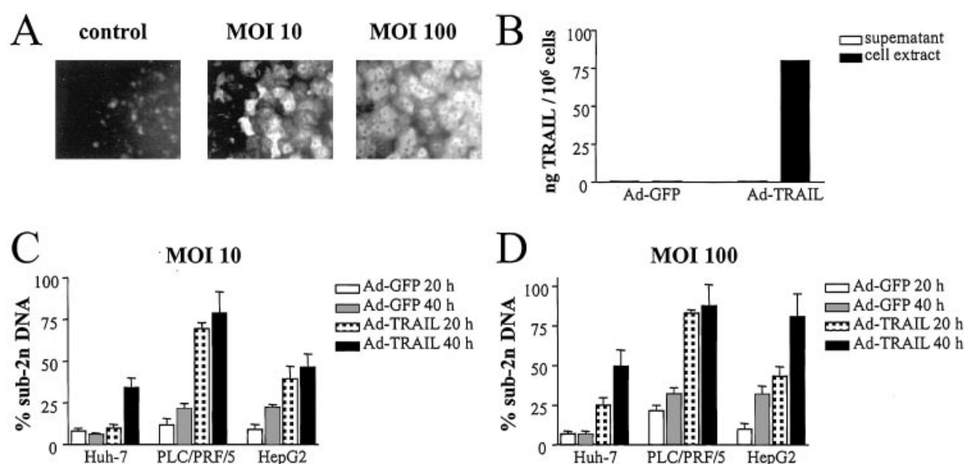

Fig. 2. Intracellular TRAIL synthesis leads to apoptosis in human hepatoma cell lines. *A,* 48 h after transduction with Ad-*TRAIL* or control vectors (*Ad-GFP*). Intracellular TRAIL synthesis could be visualized in PHHs by immunofluorescence microscopy using a monoclonal anti-TRAIL antibody. In *B,* a TRAIL-specific ELISA was done using cellular extracts and cell supernatants of transduced Huh7 cells. The results are representative of three independent experiments. In *C* and *D,* human hepatoma cell lines were transduced with different amounts of Ad-*TRAIL* or control vectors (Ad-*GFP*). Post-transduction (20 or 40 h), either with a MOI of 10 (*C*) or 100 (*D*), the fractions of apoptotic cells were determined by FACS analysis (sub-2n DNA). Shown are the mean of two independent experiments, including single SD.

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hepatoma-derived cell lines (HepG2, Huh7, and PLC/PRF/5) led to similar results (data not shown). Furthermore, by using the whole coding sequence of TRAIL, the resulting protein should be a Type II membrane-bound protein. To evaluate whether a detectable amount of membrane-bound TRAIL is cleaved and released into the supernatant, we performed an ELISA of cellular extracts and culture supernatants using transduced Huh7 cells. As a result, TRAIL could only be detected in the cellular extracts, demonstrating that no measurable release into the supernatant takes place (Fig. 2*B*).

Ad-*TRAIL* Transduction Leads to Apoptotic Cell Death in HCC Cell Lines. As a next step, we transduced HepG2, Huh7, and PLC/PRF/5 cells, with different amounts of Ad-*TRAIL* (MOI of 10 or 100). Although previous studies using cell lines from different tissues had to administer MOIs in the range of 500-1000 to get a strong apoptotic response (5, 7, 8), we unexpectedly found a strong increase in the number of apoptotic cells even by using a MOI as low as 10 (34, 79, and 47% at 40 h for Huh7, PLC/PRF/5, and HepG2 hepatoma cells, respectively; Fig. 2*C*). This apoptotic response could even be enhanced by using a MOI of 100 to 50, 88, and 81% at 40 h for Huh7, PLC/PRF/5, and HepG2 cells, respectively (Fig. 2*D*). These promising results suggested Ad-*TRAIL* transduction as a possible new strategy for the treatment of HCC.

Ad-TRAIL Transduction Leads to Caspase Activation and Apoptotic Cell Death in PHH. Systemic or intrahepatic administration of cytokines like TNF or CD95-ligand is known to cause severe liver toxicity (1). For the systemic application of sTRAIL, only a certain preparation consisting of histidine-tagged sTRAIL has been shown to selectively injure PHHs (11, 16). Because normal hepatocytes are *in situ* in close contact to HCC cells, induction of liver toxicity after an adenoviral *TRAIL* gene transfer is a key feature which has to be investigated in detail before any treatment protocols for HCC can be initiated *in vivo*.

Therefore, we transduced PHH from three different subjects with an MOI of 10 or 100 and looked for apoptotic cell death by FACS analysis. Surprisingly, we found a strong apoptotic response with 29 and 53% apoptotic cells (MOI of 10 and 100) 48-h post-transduction (Fig. 3). In control experiments, we used adenoviral vectors coding either for the reporter protein GFP (Ad-*GFP,* negative control) or for the proapoptotic CD95-ligand (Fas-ligand, positive control), which is known to induce a massive apoptotic response in hepatocytes. Moreover, to further demonstrate apoptosis induction by Ad-*TRAIL* in PHH, we looked for the activation of a downstream caspase as a surrogate marker for apoptosis. As a result, we found a strong caspase-3 activity signal for both Ad-*TRAIL* and Ad-*FasL,* even when using a relatively low MOI of 10 (Fig. 4*A*), which could not be increased significantly by using a MOI of 100 (Fig. 4*B*). The observation that treatment with Ad-*FasL* led to lower rates of caspase-3 activity could be simply explained by the fact that at the time point of examination (48-h p.i.), most Ad-*FasL*-treated cells already were dead. Taken together, Ad-*TRAIL* gene transfer to PHH results in caspase-3 activation and a substantial amount of apoptotic cells.

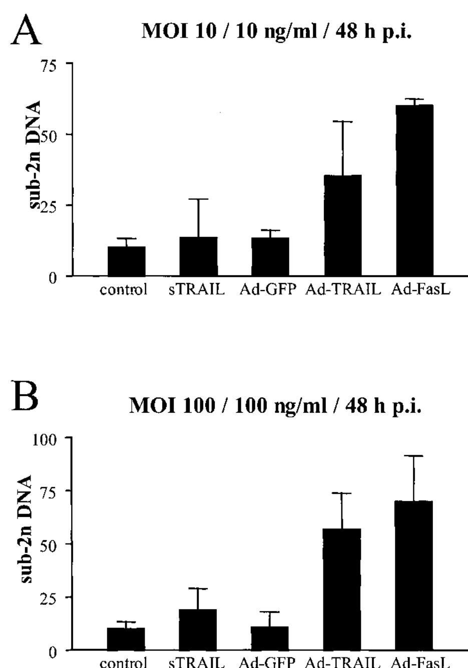

Fig. 3. Ad-*TRAIL* induces apoptosis in PHHs. Post-transduction (48 h) with one of the different adenoviruses (*Ad-GFP, -TRAIL, -FasL*) or postincubation with sTRAIL, sub-2n DNA, was determined by FACS analysis. Adenoviruses were either used with a MOI of 10 (*A*) or 100 (*B*); sTRAIL was used either with 10 ng/ml (*A*) or 100 ng/ml (*B*) in the cell supernatant. Control means no further treatment of the PHHs. Shown are the mean of three independent experiments and the corresponding single SD.

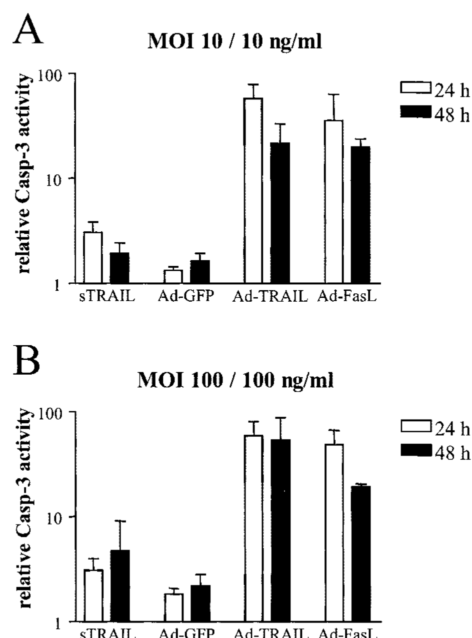

Fig. 4. Caspase-3 activation in PHHs. Caspase-3 activity in cellular extracts was determined fluorometrically. Adenoviral transduction was done with a MOI of 10 (*A*) or 100 (*B*); sTRAIL was used in the supernatant with either 10 ng/ml (*A*) or 100 ng/ml (*B*). The results are expressed as ratios of mean fluorescence compared with the activity in untreated control cells (relative caspase-3 activity). Shown are the mean of three independent experiments, including single SD.

Discussion

The liver seems to be the most important tissue concerning undesirable side effects in both the administration of adenoviral vectors (18) or approaches to treat malignancies using the cytokine TRAIL (11, 12). Therefore, each novel therapeutic strategy using these methods has to address this issue in particular (19).

First, we could show that the TRAIL apoptosis-inducing signaling pathways are in place in the investigated HCC-derived cell lines because a prolonged incubation time with high doses of sTRAIL led to an increased apoptotic response, like it has been reported for the combination of TRAIL with different chemotherapeutic agents (4). Next, we could demonstrate that the observed impaired apoptotic response of human HCC-derived cell lines could be overcome by an adenoviral-mediated gene transfer of *TRAIL.* A similar observation has been reported recently for a prostate cancer cell line, where a

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resistance to the treatment with 100 ng/ml sTRAIL could be overcome by an adenoviral gene transfer without revealing the underlying mechanisms (17). It has been suggested that an autocrine synthesis of membrane-bound TRAIL leads to the death of the transduced tumor cell being further potentiated by a potent bystander effect involving nontransduced surrounding cells (5). Concerning safety issues, adenoviral gene transfer currently cannot be restricted to one cell type only, *i.e.,* hepatocytes *versus* hepatoma cells. Therefore, adenoviral treatment of HCCs constitutes a special challenge attributable to the close neighborhood of tumor cells and hepatocytes.

To investigate the potential of liver injury induced by Ad-*TRAIL* transduction, a suitable model system has to be chosen. Experiments performed recently by Lawrence *et al.* (12) exhibited tremendous differences in the response of liver cells from different species to different human-derived TRAIL preparations. As a consequence, small animal or even primate models do not seem to be reliable in this issue. Therefore, we used cultured PHHs from different subjects in our study to monitor responses to the Ad-*TRAIL* transduction. To our knowledge, there has been only one study of *TRAIL* transduction to PHH recently, using a binary adenoviral vector system that achieves TRAIL expression in target cells only by cotransduction of the TRAIL-coding adenovirus and a second transactivating adenoviral vector (6). In that study, 31% of normal human PHHs showed signs of apoptosis 48 h after transduction, applying a very high dose of vector particles (MOI of 1000). Surprisingly, in our experiments, using a single adenoviral vector coding for full-length TRAIL, we already found a similar amount of apoptotic events using a MOI of 10, which exhibited a further increase when raising the amount of transducing particles by only the factor 10. Moreover, we could show that even when using a MOI of 10, a substantial activation of the downstream caspase-3 was detectable.

In the last 2 years, TRAIL emerged as a specific cytokine that can be used by cells of the immune system to kill virus-infected cells. A recent study by Zhang *et al.* (20) revealed that TRAIL might play a role in an unspecific immune response against adenoviral infections. In that study, recombinant adenoviruses were generated which coded for a soluble DR5-Fc molecule, being able to neutralize TRAIL. These TRAIL-inhibiting vectors were used to pretreat mice before another adenoviral vector, coding for a reporter protein, was administered. Interestingly, in that model, liver cell apoptosis after an adenoviral gene transfer of the reporter gene *lacZ* was greatly inhibited, leading to a prolonged *lacZ* gene expression (20). In respect to our study, it is tempting to speculate that the adenoviral transduction sensitizes PHH to apoptosis after a gene transfer of *TRAIL.* However, as it is shown by our transduction studies with Ad-*GFP,* adenoviral transduction alone does not lead to an apoptotic response in PHH, even when sTRAIL is supplemented to the supernatant of Ad-*GFP*-transduced cells (data not shown).

General implications for the treatment of tumors from our study are 2-fold: (*a*) for safety reasons, an application of Ad-*TRAIL in vivo* should only be done if a locally restricted application is feasible. In a recent study using a mouse model with s.c. implanted TRAIL-sensitive, HCC-derived cells, an impressing tumor response could be detected after electroporation of a gene that coded for an sTRAIL variant (10). However, even in this setting, in which the tumor and the hepatocytes were locally separated, and in addition to that, a human-derived TRAIL molecule was used in a mouse model, hepatic toxicity could be observed documented by a transient increase of liver-derived enzymes; and (*b*) another possibility to restrict *TRAIL* gene expression to malignant cells could be at the transcriptional level. An attractive example for this alternative has been shown recently by expressing the *TRAIL* gene under the control of the human telomerase reverse transcriptase promoter (6), thereby avoiding cell death in PHH, while apoptosis could be detected in malignant cells.

In conclusion, our study documents the potential of adenoviral-mediated *TRAIL* gene transfer to overcome the TRAIL insensitivity of human hepatoma cells. However, because Ad-*TRAIL* transduction of PHH resulted in a severe induction of apoptosis, any therapeutic administration of adenoviral-encoded TRAIL *in vivo* must either be strictly limited to the tumor localizations and/or restricted at the transcriptional level to avoid severe liver damage.

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References

1. Faubion, W. A., and Gores, G. J. Death receptors in liver biology and pathobiology. Hepatology, *29:* 1–4, 1999.
2. Walczak, H., Miller, R. E., Ariail, K., Gliniak, B., Griffith, T. S., Kubin, M., Chin, W., Jones, J., Woodward, A., Le, T., Smith, C., Smolak, P., Goodwin, R. G., Rauch, C. T., Schuh, J. C., and Lynch, D. H. Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand in vivo. Nat. Med., *5:* 157–163, 1999.
3. Ashkenazi, A., Pai, R. C., Fong, S., Leung, S., Lawrence, D. A., Marsters, S. A., Blackie, C., Chang, L., McMurtrey, A. E., Hebert, A., DeForge, L., Koumenis, I. L., Lewis, D., Harris, L., Bussiere, J., Koeppen, H., Shahrokh, Z., and Schwall, R. H. Safety and antitumor activity of recombinant soluble Apo2 ligand. J. Clin. Investig., *104:* 155–162, 1999.
4. Yamanaka, T., Shiraki, K., Sugimoto, K., Ito, T., Fujikawa, K., Ito, M., Takase, K., Moriyama, M., Nakano, T., and Suzuki, A. Chemotherapeutic agents augment TRAIL-induced apoptosis in human hepatocellular carcinoma cell lines. Hepatology, *32:* 482–490, 2000.
5. Kagawa, S., He, C., Gu, J., Koch, P., Rha, S. J., Roth, J. A., Curley, S. A., Stephens, L. C., and Fang, B. Antitumor activity and bystander effects of the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) gene. Cancer Res., *61:* 3330–3338, 2001.
6. Lin, T., Gu, J., Zhang, L., Huang, X., Stephens, L. C., Curley, S. A., and Fang, B. Targeted expression of green fluorescent protein/tumor necrosis factor-related apoptosis-inducing ligand fusion protein from human telomerase reverse transcriptase promoter elicits antitumor activity without toxic effects on primary human hepatocytes. Cancer Res., *62:* 3620–3625, 2002.
7. Griffith, T. S., Anderson, R. D., Davidson, B. L., Williams, R. D., and Ratliff, T. L. Adenoviral-mediated transfer of the TNF-related apoptosis-inducing ligand/Apo-2 ligand gene induces tumor cell apoptosis. J. Immunol., *165:* 2886–2894, 2000.
8. Griffith, T. S., and Broghammer, E. L. Suppression of tumor growth following intralesional therapy with TRAIL recombinant adenovirus. Mol. Ther., *4:* 257–266, 2001.
9. Lee, J., Hampl, M., Albert, P., and Fine, H. A. Antitumor activity and prolonged expression from a TRAIL-expressing adenoviral vector. Neoplasia, *4:* 312–323, 2002.
10. Yamashita, Y. I., Shimada, M., Tanaka, S., Okamamoto, M., Miyazaki, J. I., and Sugimachi, K. Electroporation-mediated tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)/Apo2L gene therapy for hepatocellular carcinoma. Hum. Gene Ther., *13:* 275–286, 2002.
11. Jo, M., Kim, T. H., Seol, D. W., Esplen, J. E., Dorko, K., Billiar, T. R., and Strom, S. C. Apoptosis induced in normal human hepatocytes by tumor necrosis factor-related apoptosis-inducing ligand. Nat. Med., *6:* 564–567, 2000.
12. Lawrence, D., Shahrokh, Z., Marsters, S., Achilles, K., Shih, D., Mounho, B., Hillan, K., Totpal, K., DeForge, L., Schow, P., Hooley, J., Sherwood, S., Pai, R., Leung, S., Khan, L., Gliniak, B., Bussiere, J., Smith, C. A., Strom, S. S., Kelley, S., Fox, J. A., Thomas, D., and Ashkenazi, A. Differential hepatocyte toxicity of recombinant Apo2L/TRAIL versions. Nat. Med., *7:* 383–385, 2001.
13. Sinzger, C., Bissinger, A. L., Viebahn, R., Oettle, H., Radke, C., Schmidt, C. A., and Jahn, G. Hepatocytes are permissive for human cytomegalovirus infection in human liver cell culture and in vivo. J. Infect. Dis., *180:* 976–986, 1999.
14. Wybranietz, W., Gross, C., Phelan, A., O'Hare, P., Spiegel, M., Graepler, F., Bitzer, M., Stahler, P., Gregor, M., and Lauer, U. Enhanced suicide gene effect by adenoviral transduction of a VP22-cytosine deaminase (CD) fusion gene. Gene Ther., *8:* 1654–1664, 2001.
15. Bitzer, M., Prinz, F., Bauer, M., Spiegel, M., Neubert, W. J., Gregor, M., Schulze-Osthoff, K., and Lauer, U. Sendai virus infection induces apoptosis through activation of caspase-8 (FLICE) and caspase-3 (CPP32). J. Virol., *73:* 702–708, 1999.
16. Ozoren, N., Kim, K., Burns, T. F., Dicker, D. T., Moscioni, A. D., and El Deiry, W. S. The caspase 9 inhibitor Z-LEHD-FMK protects human liver cells while permitting death of cancer cells exposed to tumor necrosis factor-related apoptosis-inducing ligand. Cancer Res., *60:* 6259–6265, 2000.
17. Voelkel-Johnson, C., King, D. L., and Norris, J. S. Resistance of prostate cancer cells to soluble TNF-related apoptosis-inducing ligand (TRAIL/Apo2L) can be overcome by doxorubicin or adenoviral delivery of full-length TRAIL. Cancer Gene Ther., *9:* 164–172, 2002.
18. Somia, N., and Verma, I. M. Gene therapy: trials and tribulations. Nat. Rev. Genet., *1:* 91–99, 2000.
19. Gores, G. J., and Kaufmann, S. H. Is TRAIL hepatotoxic? Hepatology, *34:* 3–6, 2001.
20. Zhang, H. G., Xie, J., Xu, L., Yang, P., Xu, X., Sun, S., Wang, Y., Curiel, D. T., Hsu, H. C., and Mountz, J. D. Hepatic DR5 induces apoptosis and limits adenovirus gene therapy product expression in the liver. J. Virol., *76:* 5692–5700, 2002.

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