Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand Activates Pro-Survival Signaling Pathways, Nuclear Factor-κB and Extracellular Signal-Regulated Kinase 1/2 in Trophoblast Cell Line, JEG-3

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ABSTRACT

Tumor necrosis factor-related apoptosis inducing ligand (TRAIL) is a well-known inducer of apoptotic cell death in many tumor cells. TRAIL is expressed in human placenta, and cytotrophoblast cells express TRAIL receptors. However, the role of TRAIL in human placentas and cytotrophoblast cells is not well understood. In this study a trophoblast cell line, JEG-3, was used as a model system to examine the effect of TRAIL on key intracellular signaling pathways involved in the control of trophoblastic cell apoptosis and survival. JEG-3 cells expressed receptors for TRAIL, death receptor (DR) 4, DR5, decoy receptor (DcR) 1 and DcR2. Recombinant human TRAIL (rhTRAIL) did not have a cytotoxic effect determined by MTT assay and did not induce apoptotic cell death determined by poly-(ADP-ribose) polymerase cleavage assay. rhTRAIL induced a rapid and transient nuclear translocation of nuclear factor-κB (NF-κB) determined by immunoblotting using nuclear protein extracts. rhTRAIL rapidly activated extracellular signal-regulated protein kinase (ERK) 1/2 as determined by immnoblotting for phospho-ERK1/2. However, c-Jun N-terminal kinase (JNK), p38 mitogen-activated protein kinase (p38MAPK) and Akt (protein kinase B) were not activated by rhTRAIL. The ability of TRAIL to induce NF-κB and ERK1/2 suggests that interaction between TRAIL and its receptors may play an important role in trophoblast cell function during pregnancy.

(Key words: Tumor necrosis factor-related apoptosis-inducing ligand, Nuclear factor-κB, Extracellular signal-regulated protein kinase 1/2, Trophoblast cell)

INTRODUCTION

The tumor necrosis factor (TNF) superfamily is involved in not only programmed cell death but also cell proliferation and differentiation (Locksley, Killeen and Lenardo, 2001), and is expressed in human placenta (Chen et al., 1991; Bamberger et al., 1997; Phillips et al., 1999; Phillips, Ni and Hunt, 2001). Among many members of the TNF superfamily, TNF-related apoptosis inducing ligand (TRAIL) has been shown to be present at the maternal-fetal interface in human (Phillips et al., 1999).

TRAIL, also known as Apo-2 ligand (Wiley et al., 1995; Pitti et al., 1996), exists as either a type II transmembrane protein or as a soluble protein by cysteine protease cleavage (Mariani and Krammer, 1998). TRAIL can interact with five receptors, i.e., death receptor 4 (DR4; also called TRAIL R1), DR5 (also called TRAIL R2), decoy receptor 1 (DcR1; also called TRAIL R3 and TRID), DcR2 (also called TRAIL R4 and TRUNDD), and a soluble receptor, osteoprotegerin (Pan et al., 1997; Ashkenazi & Dixit, 1998). DR4 and DR5 are capable of transducing an apoptotic signal upon binding to TRAIL, whereas DcR1 and DcR2 are not due to the lack of a functional death domain (Sheridan et al., 1997; Ashkenazi & Dixit, 1998). Homotrimerization of DR4 and DR5 by TRAIL binding leads to the recruitment of an adaptor molecule, Fas-associated death domain protein (FADD), on the intracellular death domain of the receptors, and induces subsequent activation caspase cascade, which leads to cleavage of the cellular substrates, including poly-(ADP-ribose) polymerase (PARP) (Ashkenazi & Dixit, 1998). However, it has also been shown that TRAIL can activate other signaling pathways, such as nuclear factor-κB (NF-κB; Chaudhary et al., 1997; Schneider et al., 1997), mitogen-activated protein kinases (MAPKs; Chou et al., 2001; Hu, Johnson and Shu, 1999; Secchiero et al., 2003, 2004) and Akt (also known as protein kinase B; Secchiero et al., 2003), which do not lead to apoptosis.

Many studies have shown that TRAIL has a unique property to induce apoptosis in a variety of tumor cells

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(Wiley et al., 1995; Pitti et al., 1996) so that it is being developed as a potential antitumor therapeutic agent (Ashkenazi et al., 1999; Walczak et al., 1999). Although the main biological function of TRAIL seems to be induction of apoptosis, the constitutive expression TRAIL and it receptor in wide range of normal tissues (Wiley et al., 1995; Pitti et al., 1996) suggests that biological roles of TRAIL may be more than inducing apoptosis of cancer cells. However, the roles of TRAIL in human placenta and the intracellular signaling pathways induced by TRAIL are largely unknown. Therefore, the purpose of this study was to gain insights into the TRAIL function in trophoblast cells and to determine how TRAIL modulates key intracellular signaling pathways which may be involved in trophoblast cell apoptosis and survival using a transformed trophoblast cell line, JEG-3.

MATERIALS AND METHODS

Reagents and Cell Lines

Recombinant human TRAIL (rhTRAIL; GF092) was purchased from Chemicon International (Temecula, CA). Antibodies directed against DR4 (PC398), DR5 (PC392), DcR1 (PC402), DcR2 (PC391) and PARP (AM30) were from Oncogene Research Products (San Diego, CA). Antibodies directed against phospho-extracellular signal-regulated kinase1/2 (phospho-ERK1/2; 9106), ERK1/2 (9102), phospho-c-Jun N-terminal kinase (phospho-JNK; 9255), JNK (9252), phospho-p38MAPK (9216), p38MAPK (9212), phospho-Akt (9271) and Akt (9272), and U0126 (9903), an MAPK/ERK1/2 (MEK1/2) inhibitor, were from Cell Signaling Technology (Beverly, MA). Antibody directed against NF-κB (p65; sc-109) was from Santa Cruz Biotechnology (Santa Cruz, CA), Nucleoporin (610497) from BD Biosciences (Bedford, MA), and Actin (A2066) from Sigma (St. Louis, MO). SN50 (P-600), an inhibitor of NF-κB nuclear translocation, was from BIOMOL (Plymouth Meeting, PA).

Trophoblast-derived choriocarcinoma cell line, JEG-3 (HTB-36), and cervical cancer cell line, HeLa (CCL-2), were purchased from the American Type Culture Collection (Manassas, VA). The cell lines were grown in tissue culture flasks at 37°C in RPMI 1640 (Sigma) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Norcross, GA), 200 mM L-glutamine, 1 mM sodium pyruvate, 50 μM 2-mercaptoethanol and antibiotics (Sigma).

Cytotoxicity Assay

JEG-3 and HeLa cells were plated at 1×10^4 cells/well into 96-well microwell plates in culture medium (RPMI 1640 containing 10% FBS, 200 mM L-glutamine, 1 mM sodium pyruvate, 50 μM 2-mercaptoethanol and antibiotics). After culture overnight at 37°C, rhTRAIL was added to a final concentration of 0, 10, 100 or 1,000 ng/ml, and cultures were continued for 24 h. Mitochondrial enzyme activity was evaluated using an MTT kit from Promega (Madison, WI), following the manufacturer's directions. Color intensity was determined spectrophotometrically at A_{560}. Each experiment was done in triplicate and was repeated three times.

Poly-(ADP-ribose) Polymerase (PARP) Cleavage Assay

JEG-3 and HeLa cells were plated in 6-well plates at 2×10^5 cells/well in 3 ml of culture medium, and cultured overnight at 37°C. On the following day, cells were washed with culture medium, treated with or without 100 ng/ml of rhTRAIL in 3 ml of culture medium, and incubated for 24 h. Proteins were then extracted from each treatment and PARP cleavage was analyzed by immunoblotting.

Protein Isolation and Immunoblot Analysis

For signaling studies, JEG-3 cell monolayer cultures were grown to 80~90% confluence on 100-mm tissue culture plates and then incubated in culture medium without serum for 24 h. Cells were treated with 100 ng/ml of rhTRAIL for the indicated time.

To obtain cellular proteins, cells were rinsed with cold Hanks' Balanced Salt Solution (HBSS; Sigma) and lysed by incubating in lysis buffer (1% Triton X-100, 0.5% Nonidet P-40, 150 mM NaCl, 10 mM Tris, 1 mM EDTA, 1 mM EGTA, 0.2 mM Na_3VO_4, 0.2 mM PMSF, 50 mM NaF, 30 mM $Na_4P_2O_7$, 1 μg/ml leupeptin, and 1 μg/ml pepstatin) for 30 min at 4°C. Cell lysates were passed through a 26-gauge needle and then clarified by centrifugation (16,000×g, 15 min, 4°C).

To analyze NF-κB nuclear translocation, nuclear protein extracts were prepared, as described previously (Zheng et al., 1993; Muroi, Muroi and Suzuki, 1994). Briefly, cells were rinsed with ice-cold HBSS, collected by scraping in 5 ml HBSS, and washed with 500 μl of cold buffer I (10 mM HEPES, 10 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 1 μg/ml pepstatin A, 10 μg/ml aprotinin, and 10 μg/ml leupeptin, pH 7.9) by centrifugation at 300×g for 10 min at 4°C. Cells were then resuspended in 200 μl of cold buffer I containing 0.5% (v/v) Nonidet P-40, incubated on ice for 15 min to swell, and centrifuged for 5 min at 2,000 rpm. The pellet was washed once with buffer I by centrifuging at 2,000 rpm for 5 min, and resuspended in 50 μl of buffer II (20 mM HEPES, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mMEDTA, 25% glycerol, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 1 μg/ml pepstatin A, 10 μg/ml aprotinin, and 10 μg/ml leupeptin, pH 7.9). The suspension was incubated on ice for 15 min with vigorous vortexing every 5 min and centrifuged at 16,000×g for 10 min, and the resulting supernatant was collected.

The concentrations of protein in cell lysates were determined using a Bradford protein assay (Bio-Rad Laboratories, Richmond, CA) with bovine serum albumin used as the standard. Proteins in cell lysates (30 μg/lane) were de-

natured in SDS-PAGE buffer, separated on 12% SDS- PAGE gels, and transferred to nitrocellulose membrane. Blot was blocked for 1 h at room temperature with 5% (w/v) nonfat milk-TBST (Tris-buffered saline with 0.1% Tween-20). Blot was then incubated overnight at 4°C with primary antibodies diluted in 2% milk-TBST. Blot was rinsed for 30 min at room temperature with TBST, incubated with the peroxidase-conjugated secondary antibodies for 1 h at room temperature, and rinsed again for 30 min at room temperature with TBST.

Immunoreactive proteins were detected by chemiluminescence (SuperSignal West Pico, Pierce Chemical Co., Rockford, IL) according to the manufacturer's recommendations using Hyperfilm ECL (Amersham Biosciences, Piscataway, NJ). Blot was reblotted with actin, nucleoporin or respective non-phosphorylated protein kinase antibodies to assess consistent loading. The optical density of protein bands in the immunoblots was quantified by scanning densitometry using EPSON1680 (EPSON, Long Beach, CA) and GelPro Analyzer (Media Cybernetics, Silver Spring, MD). Values are presented as the ratio of each phospho-protein kinase integrated optical density (IOD) to non-phospho-protein kinase IOD, or as the ratio of NF-κB IOD to nucleoporin IOD.

Statistical Analysis

Data from cytotoxicity assay were subjected to least-squares ANOVA using the general linear models (GLM) procedures of the Statistical Analysis System (SAS, Cary, NC), followed by preplanned contrasts (0 vs. 10; 0 vs. 100; 0 vs. 1000) to test for effects of treatments. All tests of statistical significance were performed using the appropriate error terms according to the expectation of mean squares. Data are presented as least-squares means (LSM) with standard errors (SE).

RESULTS

Expression of TRAIL Receptors in JEG-3 Cells

Since TRAIL can bind to four different membrane receptors (Ashkenazi and Dixit, 1998), we have analyzed whether JEG-3 cells express membrane-bound receptors for TRAIL by immunoblotting. As shown in Fig. 1, all membrane-bound TRAIL receptors were detectable in JEG-3 cells as well as in HeLa cells for DR4, DR5 and DcR2 (Sheridan et al., 1997; Bernard et al., 2001; Ravi et al., 2001), and HT29 cells for DcR1 (Fiorucci et al., 2001) as positive con-trols.

TRAIL does not Induce Apoptotic Cell Death in JEG-3 Cells

Having observed that JEG-3 cells express receptors for

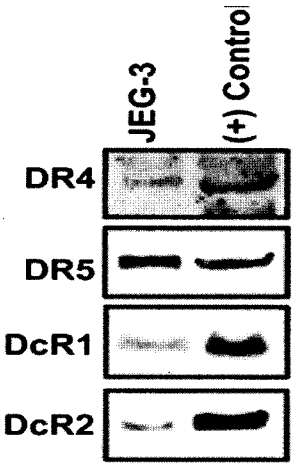

Fig. 1. Expression of TRAIL receptors in JEG-3 cells. Total cellular lysates from JEG-3 cells, and positive (+) control cells, HeLa (DR4, DR5 and DcR2) and HT29 (DcR1) cells were analyzed by immunoblotting.

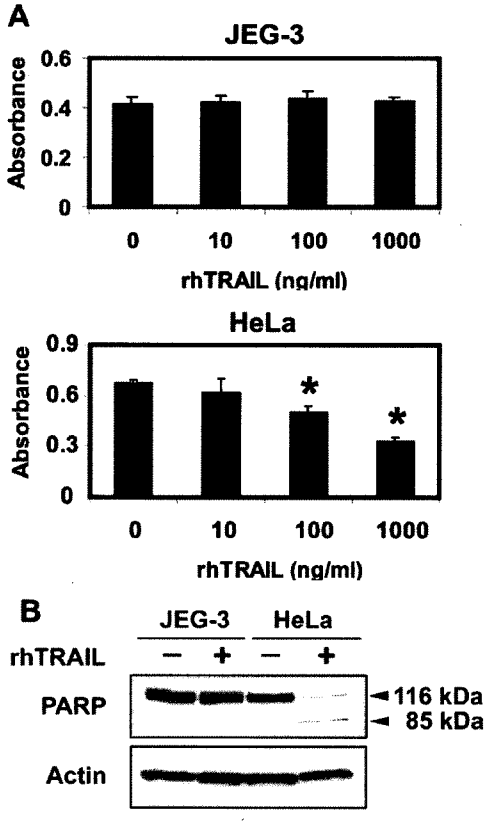

Fig. 2. Effects of TRAIL on cytotoxicity of JEG-3 cells (A) and PARP cleavage (B). Cells were treated with recombinant human TRAIL (rhTRAIL) for 24 h, and cytotoxicity was analyzed by MTT assay and PARP cleavage by immunoblotting. *, $P < 0.01$ when compared to control (0 ng/ml).

TRAIL, including DR4 and DR5, we determined if TRAIL can induce cell death in JEG-3 cells by MTT assay and PARP cleavage assay (Fig. 2). When cells were treated for 24 h with

rhTRAIL, rhTRAIL induced cytotoxicity in HeLa cells ($p < 0.01$), used as a positive control for TRAIL-induced cytotoxicity, but it did not induce cytotoxicity ($p > 0.05$) in JEG-3 cells (Fig. 2A). As expected from cytotoxicity assay, rhTRAIL did not induce PARP cleavage in JEG-3 cells, which is an evidence for the activation of apoptotic signal pathway (Soldani and Scovassi, 2002), whereas it inducedPARP cleavage in HeLa cells (Fig. 2B).

TRAIL Induces NF-κB Nuclear Translocation

Having determined that TRAIL expressed receptors and did not induce apoptotic cell death in JEG-3 cells, we have analyzed whether TRAIL activated any intracellular signaling pathways.

First, we analyzed activation of NF-κB signaling pathway. Once NF-κB signaling pathway is activated, NF-κB translocates into the nucleus (Li and Verma, 2002). Thus, we analyzed nuclear levels of NF-κB (p65) by immunoblotting after treating cells with rhTRAIL for 0, 5, 15, 30, 60 or 120 min. As shown in Fig. 3A, rhTRAIL increased nuclear levels of NF-κB in 5 min of treatment, reaching the highest levels in 15 min and decreasing thereafter. Nuclear translocation of NF-κB was decreased by pretreatment of SN50, an inhibitor of NF-κB nuclear translocation, for 30 min followed by rhTRAIL treatment for 15 min (Fig. 3B).

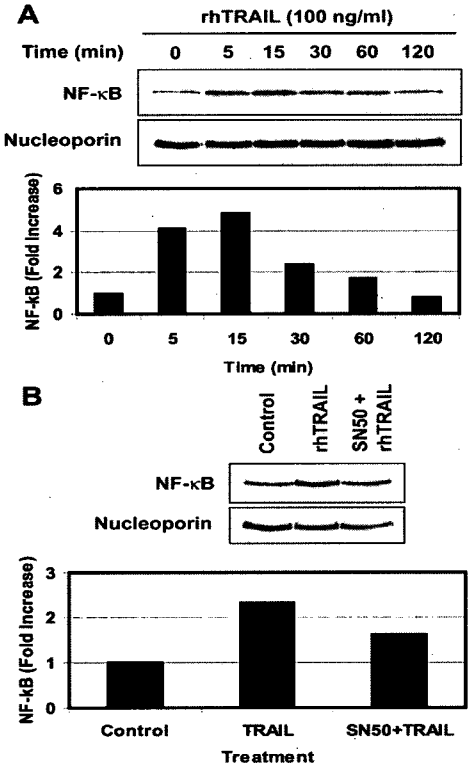

Fig. 3. Activation of NF-κB by TRAIL in JEG-3 cells. A. Cells were treated with rhTRAIL for indicated amount of time, and nuclear protein extracts were analyzed for NF-κB by immunoblotting. B. Cells were non-treated (control), TRAIL or pretreated with SN50 for 30 min and then treated with TRAIL for 15 min, and nuclear protein extracts were analyzed for NF-κB. Nucleoporin was used for loading control. The ratio of NF-κB density to nucleoporin density obtained by densitometry is shown. A representative of three separate experiments with similar results is shown.

TRAIL Activates ERK1/2 but not JNK, p38MAPK and Akt

We next investigated whether TRAIL activates MAPK pathways, ERK1/2, JNK and p38MAPK, and Akt pathway in JEG-3 cells. Since activation of MAPKs, ERK1/2, JNK and p38MAPK, and Akt are achieved by phosphorylation on kinases, specific antibodies, which detect phosphorylated residues upon activation, were used to assess activation of each kinase after rhTRAIL treatment (Fig. 4).

As shown in Fig. 4A, rhTRAIL induced ERK1/2 activation in 5 min of rhTRAIL treatment. Similarly to NF-κB activation after rhTRAIL treatment, ERK1/2 activation reached the highest levels in 15 min and decreased thereafter. Activation of ERK1/2 was inhibited by pretreatment of U0126, an inhibitor of MEK1/2, which is an upstream kinase of ERK1/2, for 30 min followed by rhTRAIL treatment for 15 min (Fig. 4B).

We then determined whether other MAPKs, JNK and p38MAPK, and Akt were activated by TRAIL in JEG-3 cells. As shown in Fig. 5, rhTRAIL did not induced activation of JNK, p38MAPK and Akt.

DISCUSSION

The result of this study using a trophoblast cell line, JEG-3, show that (1) TRAIL does not induce apoptotic cell death; (2) TRAIL induces rapid nuclear translocation of NF-κB and phosphorylation of ERK1/2; and (3) TRAIL does not induce activation of JNK, p38MAPK and Akt.

TRAIL is a well-known inducer of apoptosis (Ashkenazi and Dixit, 1998). TRAIL expression has been shown in human placenta (Phillips et al., 1999), and expression of TRAIL receptors has been determined in cytotrophoblast cells (Phillips et al., 1999; Ka, unpublished results), suggesting that TRAIL and its receptor system may affect cytotrophoblast cell function. Upon TRAIL binding, death-inducing receptors, DR4 and DR5, recruits FADD to the receptors, which in turn recruits and activates caspase-8. Caspase-8 propagates apoptosis signaling pathway by activating caspase-3, a key executioner caspase responsible for proteolytic cleavage of many proteins, including PARP (Cohen, 1997; Lazebnik et al., 1994). In this study, TRAIL did not induce cytotoxicity and PARP cleavage in JEG-3, which expressed death receptors, whereas TRAIL induced cytotoxicity and PARP cleavage in HeLa cells causing apoptotic cell death (Phillips et al., 1999). Although TRAIL can induce apoptosis in many tumor cell types, some tumor cells are resistant to TRAIL-induced apoptosis (Franco et al.,

2001; Trauzold et al., 2001; Oya et al., 2001). It has been suggested that expression of decoy receptors and intracellular apoptosis inhibitors is responsible for the resistance to TRAIL-induced apoptosis (Pan et al., 1997; Sheridan et al., 1997; Thome et al., 1997; Leverkus et al., 2003). In JEG-3 cells as well as in primary cytotrophoblast cells, expression of decoy receptors, DcR1 and DcR2, inhibitors of apoptosis (IAPs; Ka and Hunt, 2003) and FLICE-like inhibitory protein (FLIP; Ka and Hunt, unpublished results) has been determined. Thus, expression of DcR1, DcR2 and intracellular apoptosis inhibitors such as IAPs and FLIP may be responsible for protecting JEG-3 cells from undergoing apoptosis by TRAIL, although the detailed mechanism by which JEG-3 cells protect from undergoing apoptosis by TARIL remains to be determined.

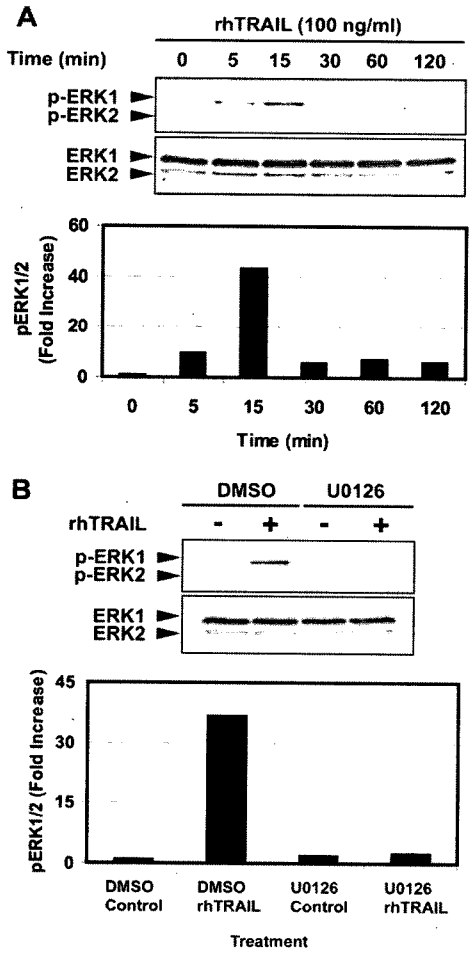

Fig. 4. Activation of ERK1/2 by TRAIL in JEG-3 cells. A. Cells were treated with rhTRAIL for indicated amount of time, and total cellular protein extracts were analyzed for ERK1/2 and pERK1/2 by immunoblotting. B. Cells were pretreated with DMSO or an MEK1/2 inhibitor, U0126 (0 or 50 μM) for 30 min, treated with rhTRAIL (100 ng/ml) for 15 min and analyzed for ERK1/2 and pERK1/2 by immunoblotting. ERK1/2 was used for loading control. The ratio of pERK1/2 to ERK1/2 density obtained by densitometry is shown. A representative of three separate experiments with similar results is shown.

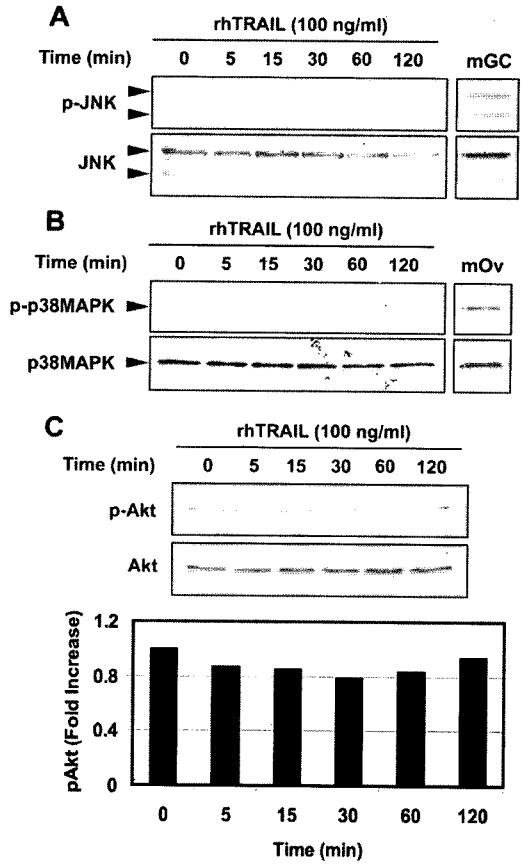

Fig. 5. Activation of JNK (A), p38MAPK (B) and Akt (C) by TRAIL in JEG-3 cells. Cells were treated with rhTRAIL for indicated amount of time, and total cellular protein extracts were analyzed for pJNK, JNK, p-p38MAPK, p38MAPK, pAkt and Akt by immunoblotting. Mouse granulose cell extracts (mGC) and mouse ovarian tissue extracts (mOv) served as positive control for pJNK and p-p38MAPK, respectively. The ratio of pAkt to Akt density obtained by densitometry is shown. A representative of three separate experiments with similar results is shown.

In spite of little information on nonapoptotic function of TRAIL, there is some evidence that TRAIL induces proliferation and production of cytokines (Chou et al., 2001; Secchiero et al., 2003), and these may be mediated by nonapoptotic signaling pathways such as NF-κB (Degli-Esposti et al., 1997; Chaudhary et al., 1997; Schneider et al., 1997), MAPKs (Hu, Johnson and Shu, 1999; Chou et al., 2001; Milani et al., 2003; Secchiero et al., 2003) and Akt (Secchiero et al., 2003).

NF-κB activation by TRAIL is mediated through DR4, DR5 and DcR2 (Chaudhary et al., 1997; Schneider et al., 1997). NF-κB represents a group of structurally related and evolutionarily conserved transcription factors, which plays a critical role in immune responses (Ghosh and Karin, 2002; Li and Verma, 2002). NF-κB is present in the cytoplasm in association with inhibitors of NF-κB (IκBs). Once the signaling pathway is activated by various inducers, IκB becomes phosphorylated and degraded, allowing NF-κB to translocate into the nucleus and to activate transcription

of a variety of gene, including cytokines, chemokines and antiapoptotic factors (Ghosh and Karin, 2002; Li and Verma, 2002). In this study, TRAIL induced NF-κB nuclear translocation, and NF-κB activation was transient, in accordance with other reports that nuclear translocation of NF-κB is transient and dynamically regulated (Ghosh and Karin, 2002; Li and Verma, 2002; Hoffmann et al., 2002).

NF-κB has been suggested to protect cells from undergoing apotosis (Ghosh and Karin, 2002; Li and Verma, 2002). In some types of cells the apoptosis-inducing TNF superfamily ligands activate NF-κB, and cells that respond in this manner do not undergo apoptosis (Ashkenazi and Dixit, 1998). Since it has been shown that NF-κB induces expression of antiapoptotic factors such as IAPs and FLIP (Ghosh and Karin, 2002; Li and Verma, 2002), which are present in trophoblast cells, it seems to be reasonable to propose that NF-κB signaling pathway may also be responsible to protect cells from TRAIL-induced apoptosis in JEG-3 cells.

MAPKs are a family of serine-threonine protein kinases, which is conserved through evolution, and three major MAPK families, ERK1/2, JNK and p38MAPK, have been identified in mammalian cells (Pearson et al., 2001). MAPKs regulate cell proliferation, differentiation and survival (Pearson et al., 2001). In human placenta, ERK1/2 are expressed in villous and extravillous cytotrophoblast cells. In villous cytotrophoblast cells activated (phosphorylated) ERK1/2 are detectable only in the first, while in extravillous cytotrophoblast cells activated ERK1/2 are detectable during whole gestation period (Kita et al., 2003).

Activation of MAPK by TRAIL has been shown in various types of cells (Hu, Johnson and Shu, 1999; Chou et al., 2001; Milani et al., 2003; Secchiero et al., 2003). TRAIL activates ERK1/2 in human vascular endothelial cells (Secchiero et al., 2003) and SK-N-MC neuroblastoma cell (Milani et al., 2003), mediating cell survival and proliferation. JNK activation in human embryonic kidney 293 cells (Hu, Johnson and Shu, 1999) and p38MAPk activation in mouse T cells (Chou et al., 2001) by TRAIL were also determined. In JEG-3 cells, TRAIL activated ERK1/2, but not JNK and p38MAPK. Although TRAIL did not induce cell proliferation in this cell, TRAIL may induce other cellular function through ERK1/2 pathway. And, it is also possible that during pregnancy TRAIL affects functions of cytotrophoblast cells, which express ERK1/2.

Akt is a serine-threonine kinase that acts on cell proliferation and inhibition of apoptosis (Franke et al., 2003). Activation of Akt pathway by TRAIL has been documented (Secchiero et al., 2003), but TRAIL did not activate Akt in JEG-3 cells, suggesting that activation of intracellular signaling pathways by TRAIL is cell-type specific.

In conclusion, our results show that TRAIL does not induce apoptosis in JEG-3 cells, but activates NF-κB and ERK1/2 signaling pathways, suggesting that TRAIL may affect other cellular functions in this trophoblast cell line, and that TRAIL may affect placental trophoblast cell function during pregnancy.

REFERENCES

1. Ashkenazi A, Dixit VM (1998): Death receptors: signaling and modulation. Science 281:1305-1308.
2. Ashkenazi A, Pai RC, Fong S, Leung S, Lawrence DA, Marsters SA, Blackie C, Chang L, McMurtrey AE, Hebert A, DeForge L, Koumenis IL, Lewis D, Harris L, Bussiere J, Koeppen H, Shahrokh Z, Schwall RH (1999): Safety and antitumor activity of recombinant soluble Apo2 ligand. J Clin Invest 104:155-162.
3. Bamberger AM, Schulte HM, Thuneke I, Erdmann I, Bamberger CM, Asa SL (1997): Expression of the apoptosis-inducing Fas ligand (FasL) in human first and third trimester placenta and choriocarcinoma cells. J Clin Endocrinol Metab 82:3173-3175.
4. Bernard D, Quatannens B, Vandenbunder B, Abbadie C (2001): Rel/NF-κB transcription factors protect against tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)-induced apoptosis by up-regulating the TRAIL decoy receptor DcR1. J Biol Chem 276:27322-27328.
5. Chaudhary PM, Eby M, Jasmin A, Bookwalter A, Murray J, Hood L (1997): Death receptor 5, a new member of the TNFR family, and DR4 induce FADD-dependent apoptosis and activate the NF-κB pathway. Immunity 7: 821-830.
6. Chen HL, Yang YP, Hu XL, Yelavarthi KK, Fishback JL, Hunt JS (1991): Tumor necrosis factor alpha mRNA and protein are present in human placental and uterine cells at early and late stages of gestation. Am J Pathol 139:327-335.
7. Chou AH, Tsai HF, Lin LL, Hsieh SL, Hsu PI, Hsu PN (2001): Enhanced proliferation and increased IFN-gamma production in T cells by signal transduced through TNF-related apoptosis-inducing ligand. J Immunol 167:1347-1352.
8. Cohen GM (1997): Caspases: the executioners of apoptosis. Biochem J 326:1-16.
9. Degli-Esposti MA, Dougall WC, Smolak PJ, Waugh JY, Smith CA, Goodwin RG (1997): The novel receptor TRAIL-R4 induces NF-κB and protects against TRAIL-mediated apoptosis, yet retains an incomplete death domain. Immunity 7:813-820.
10. Fiorucci S, Distrutti E, Ajuebor MN, Mencarelli A, Mannucci R, Palazzetti B, Del Soldato P, Morelli A, Wallace JL (2001): NO-mesalamine protects colonic epithelial cells against apoptotic damage induced by proinflammatory cytokines. Am J Physiol Gastrointest Liver Physiol 281:G654-G665.
11. Franco AV, Zhang XD, Van Berkel E, Sanders JE, Zhang XY, Thomas WD, Nguyen T, Hersey P (2001): The role of NF-κB in TNF-related apoptosis-inducing ligand (TRA-

IL)-induced apoptosis of melanoma cells. J Immunol 166:5337-5345.
12. Franke TF, Hornik CP, Segev L, Shostak GA, Sugimoto C (2003): PI3K/Akt and apoptosis: size matters. Oncogene 22:8983-8998.
13. Ghosh S, Karin M (2002): Missing pieces in the NF-κB puzzle. Cell 109:S81-S96.
14. Hu WH, Johnson H, Shu HB (1999): Tumor necrosis factor-related apoptosis-inducing ligand receptors signal NF-κB and JNK activation and apoptosis through distinct pathways. J Biol Chem 274:30603-30610.
15. Ka H, Hunt JS (2003): Temporal and spatial patterns of expression of inhibitors of apoptosis in human placentas. Am J Pathol 163:413-422.
16. Kita N, Mitsushita J, Ohira S, Takagi Y, Ashida T, Kanai M, Nikaido T, Konishi I (2003): Expression and activation of MAP kinases, ERK1/2, in the human villous trophoblasts. Placenta 24:164-172.
17. Lazebnik YA, Kaufmann SH, Desnoyers S, Poirier GG, Earnshaw WC (1994): Cleavage of poly(ADP-ribose) polymerase by a proteinase with properties like ICE. Nature 371:346-347.
18. Leverkus M, Sprick MR, Wachter T, Mengling T, Baumann B, Serfling E, Brocker EB, Goebeler M, Neumann M, Walczak H (2003): Proteasome inhibition results in TRAIL sensitization of primary keratinocytes by removing the resistance-mediating block of effector caspase maturation. Mol Cell Biol 23:777-790.
19. Li Q, Verma IM (2002): NF-κB regulation in the immune system. Nat Rev Immunol 2:725-734.
20. Locksley RM, Killeen N, Lenardo MJ (2001): The TNF and TNF receptor superfamilies: integrating mammalian biology. Cell 104:487-501.
21. Mariani SM, Krammer PH (1998): Differential regulation of TRAIL and CD95 ligand in transformed cells of the T and B lymphocyte lineage. Eur J Immunol 28:973-982.
22. Milani D, Zauli G, Rimondi E, Celeghini C, Marmiroli S, Narducci P, Capitani S, Secchiero P (2003): Tumour necrosis factor-related apoptosis-inducing ligand sequentially activates pro-survival and pro-apoptotic pathways in SK-N-MC neuronal cells. J Neurochem 86: 126-135.
23. Oya M, Ohtsubo M, Takayanagi A, Tachibana M, Shimizu N, Murai M (2001): Constitutive activation of nuclear factor-kappaB prevents TRAIL-induced apoptosis in renal cancer cells. Oncogene 20:3888-3896.
24. Pan G, Ni J, Wei YF, Yu G, Gentz R, Dixit VM (1997): An antagonist decoy receptor and a death domain-containing receptor for TRAIL. Science 277:815-818.
25. Pearson G, Robinson F, Beers Gibson T, Xu BE, Karandikar M, Berman K, Cobb MH (2001): Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. Endocr Rev 22:153-183.
26. Phillips TA, Ni J, Pan G, Ruben SM, Wei YF, Pace JL, Hunt JS (1999): TRAIL (Apo-2L) and TRAIL receptors in human placentas: implications for immune privilege. J Immunol 162:6053-6059.
27. Phillips TA, Ni J, Hunt JS (2001): Death-inducing tumour necrosis factor (TNF) superfamily ligands and receptors are transcribed in human placentae, cytotrophoblasts, placental macrophages and placental cell lines. Placenta 22:663-672.
28. Pitti RM, Marsters SA, Ruppert S, Donahue CJ, Moore A, Ashkenazi A (1996): Induction of apoptosis by Apo-2 ligand, a new member of the tumor necrosis factor cytokine family. J Biol Chem 271:12687-12690.
29. Ravi R, Bedi GC, Engstrom LW, Zeng Q, Mookerjee B, Gelinas C, Fuchs EJ, Bedi A (2001): Regulation of death receptor expression and TRAIL/Apo2L-induced apoptosis by NF-κB. Nat Cell Biol 3:409-416.
30. Schneider P, Thome M, Burns K, Bodmer JL, Hofmann K, Kataoka T, Holler N, Tschopp J (1997): TRAIL receptors 1 (DR4) and 2 (DR5) signal FADD-dependent apoptosis and activate NF-κB. Immunity 7:831-836.
31. Secchiero P, Gonelli A, Carnevale E, Milani D, Pandolfi A, Zella D, Zauli G (2003): TRAIL promotes the survival and proliferation of primary human vascular endothelial cells by activating the Akt and ERK pathways. Circulation 107:2250-2256.
32. Secchiero P, Melloni E, Heikinheimo M, Mannisto S, Di Pietro R, Iacone A, Zauli G (2004): TRAIL regulates normal erythroid maturation through an ERK-dependent pathway. Blood 103:517-522.
33. Sheridan JP, Marsters SA, Pitti RM, Gurney A, Skubatch M, Baldwin D, Ramakrishnan L, Gray CL, Baker K, Wood WI, Goddard AD, Godowski P, Ashkenazi A (1997) Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors. Science 277:818-821.
34. Soldani C, Scovassi AI (2002): Poly(ADP-ribose) polymerase-1 cleavage during apoptosis: an update. Apoptosis 7:321-328.
35. Thome M, Schneider P, Hofmann K, Fickenscher H, Meinl E, Neipel F, Mattmann C, Burns K, Bodmer JL, Schroter M, Scaffidi C, Krammer PH, Peter ME, Tschopp J (1997): Viral FLICE-inhibitory proteins (FLIPs) prevent apoptosis induced by death receptors. Nature 386: 517-521.
36. Trauzold A, Wermann H, Arlt A, Schutze S, Schafer H, Oestern S, Roder C, Ungefroren H, Lampe E, Heinrich M, Walczak H, Kalthoff H (2001): CD95 and TRAIL receptor-mediated activation of protein kinase C and NF-κB contributes to apoptosis resistance in ductal pancreatic adenocarcinoma cells. Oncogene 20:4258-4269.
37. Walczak H, Miller RE, Ariail K, Gliniak B, Griffith TS, Kubin M, Chin W, Jones J, Woodward A, Le T, Smith C, Smolak P, Goodwin RG, Rauch CT, Schuh JC, Lynch DH (1999): Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand *in vivo*. Nat Med 5:157-163.

38. Wiley SR, Schooley K, Smolak PJ, Din WS, Huang CP, Nicholl JK, Sutherland GR, Smith TD, Rauch C, Smith CA, Goodwin RG (1995): Identification and characterization of a new member of the TNF family that induces apoptosis. Immunity 3:673-682.
(Received: 24 May 2005 / Accepted: 13 June 2005)