Central Role of the Scaffold Protein Tumor Necrosis Factor Receptor-associated Factor 2 in Regulating Endoplasmic Reticulum Stress-induced Apoptosis*\(^5\)

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Claudio Mauro\(^3\), Elvira Crescenzi\(^3\), Roberta De Mattia\(^1\), Francesco Pacifico\(^1\), Stefano Mellone\(^5\), Salvatore Salzano\(^1\), Cristiana de Luca\(^3\), Luciano D’Adamo\(^6\), Giuseppe Palumbo\(^1\), Silvestro Formisano\(^5\), Pasquale Vito\(^**\), and Antonio Leonard\(^1,2\)

From the Dipartimento di \(^4\)Biologia e Patologia Cellulare e Molecolare and \(^1\)Biochimica e Biotecnologie Mediche, Università degli Studi di Napoli “Federico II,” Via Pansini 5, 80131 Naples, Italy, the \(^2\)Istituto di Endocrinologia e Oncologia Sperimentale, CNR, Via Pansini 5, 80131 Naples, Italy, \(^3\)Dipartimento di Scienze Biologiche e Ambientali, Università degli Studi del Sannio, Via Port’Arsa 11, 82100 Benevento, Italy, and the \(^5\)Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York 10461

The endoplasmic reticulum represents the quality control site of the cell for folding and assembly of cargo proteins. A variety of conditions can alter the ability of the endoplasmic reticulum (ER) to properly fold proteins, thus resulting in ER stress. Cells respond to ER stress by activating different signal transduction pathways leading to increased transcription of chaperone genes, decreased protein synthesis, and eventually to apoptosis. In the present paper we analyzed the role that the adaptor protein tumor necrosis factor receptor-associated factor 2 (TRAF2) plays in regulating cellular responses to apoptotic stimuli from the endoplasmic reticulum. Mouse embryonic fibroblasts derived from TRAF2\(^−/−\) mice were more susceptible to apoptosis induced by ER stress than the wild type counterpart. This increased susceptibility to ER stress-induced apoptosis was because of an increased accumulation of reactive oxygen species following ER stress, and was abolished by the use of antioxidant. In addition, we demonstrated that the NF-κB pathway protects cells from ER stress-induced apoptosis, controlling ROS accumulation. Our results underscore the involvement of TRAF2 in regulating ER stress responses and the role of NF-κB in protecting cells from ER stress-induced apoptosis.

In eukaryotic cells, proteins must be correctly folded and assembled before to transit to intracellular organelles and the cell surface (1, 2). A number of cellular stress conditions can interfere with protein folding, leading to accumulation of unfolded or misfolded proteins in the endoplasmic reticulum (ER)\(^3\) lumen. The ER has evolved specific signaling pathway to deal with the potential danger represented by the misfolded proteins. This adaptive response is named unfolded protein response (3). Activation of unfolded protein response results in attenuation of protein synthesis, and up-regulation of genes encoding chaperones that facilitate the protein folding process in the ER. Thus, unfolded protein response reduces accumulation and aggregation of malfolded proteins, giving the cell the possibility of correcting the environment inside the ER (3, 4). However, if the damage is too strong and homeostasis cannot be restored, the mammalian unfolded protein response initiates apoptosis. In mammalian cells, three transmembrane proteins Ire1\(^α\) (5), Ire1\(^β\) (6), and PERK (7) act as ER stress sensor proteins and play important roles in transducing the stress signals initiated by the accumulation of malfolded proteins from the ER to the cytoplasm and nucleus. Ire1s and PERK are kept in an inactive state through association of their N-terminal lumen domain with the chaperone BiP. Following accumulation of malfolded proteins in the lumen of the ER, BiP dissociates to bind the malfolded proteins and Ire1s and PERK undergo oligomerization and transphosphorylation within their cytoplasmic kinase domains (8, 9).

Other stress response pathways are activated following ER stress, such as the JNK/SAPK and NF-κB pathways (10, 11). Activation of these pathways following ER stress is mediated by the physical and functional interaction of Ire1\(^α\) and TRAF2 (10). The central role played by TRAF2 in mediating cellular response to ER stress has been proposed based upon the observation that ectopic expression of a dominant negative mutant of TRAF2 lacking the N terminus Ring finger domain, blocks ER stress-induced NF-κB and JNK/SAPK activation, and that mouse embryonic fibroblast derived from TRAF2 knock-out mice failed to activate NF-κB following ER stress (10, 11). TRAF2 was initially identified as a TNF receptor 2 interacting protein (13). Interestingly, TRAF2-deficient MEFs are very sensitive to cell death induced by TNF and other members of the TNF receptor family (14, 15). At least part of the anti-apoptotic effect of TRAF2 can be explained by its function as a mediator of NF-κB activation, thus leading to NF-κB-dependent expression of anti-apoptotic genes. The anti-apoptotic activity of NF-κB also involves inhibition of the JNK cascade via at least two distinct mechanisms: through GADD45-β-mediated blockade of MKK7 and interference with ROS production (16, 17). It is well known that ROS or oxidative stress plays an important role in various physiological and pathological processes such as aging, inflammation, and neurodegenerative diseases (18–20). Recently, it has been demonstrated that accumulation of misfolded protein within the lumen of the ER causes accumulation of ROS and cell death (21). However, it is currently unknown whether some of the key molecules involved in ER stress response, such as TRAF2, are involved in modulation of ROS and induction of apoptosis. Here we use MEFs derived from TRAF2 knock-out mice to study the role of TRAF2...
in the regulation of pro-survival or pro-apoptotic pathways following ER stress.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Biological Reagents**—Wild type (WT) and TRAF2−/− murine embryonic fibroblasts (MEFs) were provided by Drs. T. W. Mak and W. C. Yeh (14). WT and JNK1/2−/− and WT and p65−/− MEFs were provided by Dr. R. Davis and Dr. G. Franzoso, respectively (22, 23). Cells were cultured in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal calf serum, 100 units/ml penicillin, and 100 µg/ml streptomycin. Thapsigargin was from Calbiochem and used at 5–50 nM; tunicamycin was purchased from Roche and used at 50–150 ng/ml. Dichlorodihydrofluorescein diacetate (H2DCFDA) (Calbiochem) was dissolved in Me2SO and used at 5 µM; L-NAC was dissolved in sterile water and used at 5 mM. Anti-TRAF2, anti-1-xB, and anti-JNK antibodies were purchased from Santa Cruz Biotechnology. The TRAF2 full-length expression vector was previously described (24).

**Western Blot Analysis**—Subconfluent monolayer of murine embryonic fibroblasts were washed with phosphate-buffered saline and then lysed in a lysis buffer containing 20 mM HEPES, pH 7.5, 150 mM NaCl, 10% glycerol, 1% Triton X-100, supplemented with a mixture of protease inhibitors (Roche). Equal amounts of total proteins (50 µg) were resolved by SDS-polyacrylamide gels. Separated proteins were transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA) at 4 °C for Western blot analysis. Filters were blocked for 1 h at room temperature with 10% nonfat dry milk in TBS-T buffer (10 mM Tris-HCl, pH 8, 150 mM NaCl, 0.1% Tween 20). Then, filters were probed with specific antibodies in the same buffer for 14–16 h at 4 °C. After TBS-T washing to remove excess primary antibodies, the blots were incubated in horseradish peroxidase-coupled secondary antibody for 1 h followed by enhanced chemiluminescence detection of the proteins with Hyper-film ECL detection (Amersham Biosciences).

**Luciferase Assay**—For luciferase assay, WT, TRAF2−/−, and TRAF2FL MEFs (4 × 10^4 cells per well) were seeded in 6-well (35 mm) plates. After 12 h cells were transfected with 0.5 µg of Ig-kB-LUC reporter gene plasmid using Lipofectamine. Cells were stimulated with thapsigargin or tunicamycin for 4 h, and reporter gene activity was determined by the luciferase assay system (Promega). A pRSV-B-galactosidase vector (0.2 µg) was used to normalize for transfection efficiencies.

**Retroviral Infection**—Full-length hemagglutinin-tagged TRAF2 was subcloned into the retroviral expression vector pBMN by standard cloning techniques. pBMN vector was then transfected in a packaging cell line using Lipofectamine. 48 h after transfection, the viral supernatants were supplemented with Polybrene (9 mg/ml) and filtered through a 0.45-mm filter. TRAF2−/− fibroblasts (1 × 10^6) were incubated with viral supernatants for 48 h. The expression of exogenous protein was assayed by Western blot analysis on total cell extracts using anti-TRAF2 antibodies.

**ER Stress Induction and Measurements of Apoptosis**—5 × 10^5 cells/well were seeded in 96-well culture plates and incubated for 24 or 48 h at 37 °C with different concentrations of thapsigargin or tunicamycin. Cell survival was examined using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) and an electron coupling reagent (phenazine methosulfate), according to the manufacturer’s instructions (Promega). Cell death was assessed by staining the exposed phosphatidylserine on cell membranes with fluorescein isothiocyanate-conjugated annexin V (BD Pharmingen), or propidium iodide staining according to Nicoletti et al. (25).

**Measurement of ROS Production**—Reactive oxygen species were detected with H2DCFDA (Calbiochem). H2DCFDA diffuses into the cells where it is converted into a non-fluorescent derivative (H2DCF) by endogenous esterases. H2DCF is oxidized to green fluorescent DCF in the presence of intracellular ROS. Cells were routinely treated with either tunicamycin or thapsigargin for 24 or 48 h, washed, and incubated at 37 °C for 30 min in the presence of H2DCFDA in serum-free medium. Me2SO-treated cells were used as controls. After incubation, cells were washed twice with phosphate-buffered saline, resuspended in phosphate-buffered saline, and analyzed by flow cytometry using a FACScan Cell Scanner (BD Biosciences).

**Kinase Assay**—JNK immunoprecipitates were used for the immune complex kinase assay that was performed at 30 °C for 10 min with 2 µg of substrate, 10 µCi of [γ-32P]ATP in a total of 20 µl of kinase buffer (20 mM HEPEs, pH 7.4, 10 mM MgCl2, 25 mM β-glycerophosphate, 50 mM Na3VO4, and 50 mM dithiothreitol). The substrate was glutathione S-transferase-c-Jun (amino acids 1–79). The reaction was terminated by boiling in SDS sample buffer, and the products were resolved by 12% SDS-PAGE. Phosphorylated proteins were detected by autoradiography.

**RESULTS**

**Increased Susceptibility of TRAF2−/− MEFs to ER Stress-induced Apoptosis**—TRAF2 is a scaffold protein that transduces signals from membrane receptors and the ER membrane (10–12). To assess the role of TRAF2 in apoptosis induced by ER stress, we treated MEFs derived from TRAF2−/− mice and WT MEFs with increasing concentrations of thapsigargin and tunicamycin. Both drugs induce ER stress by inhibiting ER-resident Ca2⁺-ATPase, and N-glycosylation, respectively. After a 48-h treatment, some morphological changes were observed. In particular, WT MEFs showed an extended shape, typical of cellular stress response, whereas TRAF2−/− MEFs appeared detached and shrunken (Fig. 1A). Because these morphological changes were reminiscent of apoptosis, we performed annexin V staining on WT and TRAF2−/− MEFs. As shown in Fig. 1B, treatment with thapsigargin or tunicamycin caused a dramatic increase in apoptosis in TRAF2−/− MEFs but not in WT MEFs. The higher sensitivity to apoptosis observed in TRAF2−/− MEFs was not because of an intrinsic defect of these cells, given that reintroduction of TRAF2 (TRAF2FL) completely rescued cell viability (Fig. 1C–E). TRAF2−/− MEFs showed the same susceptibility as WT MEFs to serum starvation- and doxorubicin-induced cell death (Fig. 1F). These results suggest a specific role for TRAF2 in modulating survival signals from the ER.

**ROS Mediate Increased Apoptosis in TRAF2−/− MEFs**—ER stress has recently been shown to promote oxidative stress and apoptosis (21). Hence, to have some insight on the molecular mechanism determining the increased susceptibility to ER stress-induced apoptosis, we compared ROS production in WT and TRAF2−/− MEFs. As shown in Fig. 2, treatment with thapsigargin or tunicamycin caused an increase in ROS production in TRAF2−/− MEFs but not in WT. Reconstitution of these cells with TRAF2 (TRAF2FL) blocked ROS accumulation following treatment with thapsigargin and tunicamycin (Fig. 2A and B). To investigate whether the increased production of ROS was responsible for the susceptibility of TRAF2−/− MEFs to ER stress-induced apoptosis, TRAF2−/− MEFs were treated with thapsigargin or tunicamycin in the presence of different antioxidants and 48 h later cell viability was measured by MTS assay and the ROS level by flow cytometry. As shown in Fig. 3, NAC abolished ROS accumulation and protected these cells from...
FIGURE 1. ER stress causes apoptosis in TRAF2−/− MEFs. A, WT and TRAF2−/− MEFs were treated with 100 ng/ml tunicamycin or vehicle for 48 h. Cell death was examined by morphological changes under a phase-contrast microscope. B, WT and TRAF2−/− MEFs were treated with 5 nM thapsigargin or 50 ng/ml tunicamycin for 48 h. Apoptosis was assessed by flow cytometry after staining with fluorescein isothiocyanate-conjugated annexin V. Percentage of the apoptotic cell is indicated. C, restoration of TRAF2 protein expression. TRAF2−/− MEFs were infected with an expression vector encoding full-length TRAF2. Expression of the TRAF2 protein was assessed by Western blot in WT, TRAF2−/−, and TRAF2-reconstituted cells (TRAF2FL). D, restoration of TRAF2 protein expression rescues TRAF2−/− cells from ER stress-induced apoptosis. WT, TRAF2−/−, and TRAF2FL MEFs were treated with Me2SO (Co), tunicamycin (Tun), or thapsigargin (Thaps) for 48 h. Cell viability was assessed by MTS assay. Data are mean ± S.D. from five independent experiments. Statistical analysis was by unpaired Student’s t test: **, p < 0.001; ***, p < 0.0001. E, restoration of TRAF2 protein expression rescues TRAF2−/− cells from endoplasmic reticulum stress-induced apoptosis. TRAF2−/−, WT, and TRAF2FL MEFs were treated with Me2SO (Co), 20 nM thapsigargin, or 150 ng/ml tunicamycin for 48 h and analyzed by flow cytometry. Percentage of sub-G0 cells is indicated. F, TRAF2−/− and WT MEFs were serum starved for 24 and 48 h, or treated with 0.2 μM doxorubicin for 24 and 48 h, and cell viability was assessed by MTS assay. Data are mean ± S.D. from three independent experiments. Statistical analysis was by the unpaired Student’s t test: **, p < 0.002; *** , p < 0.0001. KO, knock-out.
apoptosis. Similar results were obtained by using dithiothreitol as antioxidant (data not shown). Interestingly, also the small percentage of WT MEFs and reconstituted TRAF2−/−/H11002−/−/H11002 MEFs undergoing apoptosis following treatment with tunicamycin and thapsigargin were almost completely protected by both antioxidants (Fig. 3 and data not shown). These results demonstrated that susceptibility of TRAF2−/−/H11002−/−/H11002 MEFs to ER stress-induced apoptosis was because of increased accumulation of ROS. It is worth noting that in TRAF2−/− cells, higher levels of ROS and apoptosis were detected, even in the absence of ER stressing agents (Fig. 1, B and E, and data not shown).

TRAF2-mediated NF-κB Activation Protects Cells from ER Stress-induced Apoptosis—Given the central role played by TRAF2 to correctly signal activation of NF-κB and JNK from ER, we investigated which of these pathways control ROS accumulation and protect cells from ER stress-induced apoptosis. MEFs derived from p65 knock-out and JNK1/2 double knock-out mice were treated with thapsigargin or tunicamycin in the presence or absence of NAC. As shown in Fig. 4A, p65−/− MEFs showed very high levels of ROS following treatment with thapsigargin and tunicamycin. As expected, treatment with NAC decreased ROS accumulation by about 40%. In contrast, JNK1/2−/− MEFs showed an accumulation of ROS similar to WT MEFs (Fig. 4A). Statistical analysis is reported in Fig. 4B. We next investigated the susceptibility of p65−/− and JNK1/2−/− MEFs to apoptosis induced by thapsigargin or tunicamycin in the presence or absence of NAC. As shown in Fig. 4C, p65−/− MEFs were highly susceptible to apoptosis compared with WT MEF and treatment with NAC significantly increased cell viability. JNK1/2−/− MEFs did not show susceptibility to ER stress-induced cell death, as compared with WT MEFs. These results suggest that NF-κB protects cells from ER stress-induced apoptosis by controlling ROS accumulation.
FIGURE 3. ROS production correlates with endoplasmic reticulum stress-induced apoptosis. WT, TRAF2−/−, and TRAF2FL MEFs were treated with Me2SO (Co), 150 ng/ml tunicamycin (Tun), or 20 nM thapsigargin (Thaps) for 48 h, in the presence or absence of antioxidants (NAC or dithiothreitol). ROS production was assessed by flow cytometry after labeling with H2DCFDA. Cell viability was evaluated by MTS assay. Data are mean ± S.D. from three independent experiments. Statistical analysis was by the unpaired Student’s t test: *, p < 0.02; **, p < 0.002; ***, p < 0.0001.
FIGURE 4. Differential susceptibility of p65<sup>−/−</sup> and JNK1/2<sup>−/−</sup> MEFs to endoplasmic reticulum-dependent oxidative stress. A, JNK1/2<sup>−/−</sup> and p65<sup>−/−</sup> MEFs were treated with Me<sub>2</sub>S0 (Co), 20 nM thapsigargin (Thaps), or 150 ng/ml tunicamycin (Tun) for 24 h, in the presence or absence of 5 mM NAC. ROS production was assessed by flow cytometry after labeling with H<sub>2</sub>DCFDA. B, Kolmogorov-Smirnov statistical analysis of flow cytometric data were used according to Cell Quest Software (BD Biosciences). D values by Kolmogorov-Smirnov analysis (p < 0.001) are shown. C, p65<sup>−/−</sup> and JNK1/2<sup>−/−</sup> MEFs were treated with Me<sub>2</sub>S0 (Co), thapsigargin (Thaps), or tunicamycin (Tun) for 24 h, in the presence or absence of 5 mM dithiothreitol. Cell viability was evaluated by MTS assay. Data are mean ± S.D. from three independent experiments. Statistical analysis was by the unpaired Student’s t test: *, p < 0.02; **, p < 0.002. KO, knock-out.

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To have further insight on the cross-talk between the NF-κB and the JNK pathways after ER stress, we evaluated activation of both pathways in WT, TRAF22/−, and reconstituted TRAF2FL MEFs. Treatment with tunicamycin caused activation of NF-κB in WT and TRAF2FL MEFs, as demonstrated by the disappearance of the inhibitory subunit IκBα (Fig. 5A) and by the increased activity of a κB-driven luciferase reporter gene (Fig. 5B). In the absence of TRAF2 it was not possible to detect activation of NF-κB. The observed activation of NF-κB was functional as demonstrated by the reappearance of the inhibitory subunit IκBα, a known early target gene of NF-κB (Fig. 5A).

Activation of JNK in WT and TRAF2FL MEFs stimulated with tunicamycin was detected 90 min after stimulation and decreased thereafter. Treatment with antioxidant did not affect JNK activation (Fig. 6). In contrast, in TRAF22/− MEFs, activation of JNK was detectable only 6 h after stimulation and remained sustained for up to 12 h. This sustained activation of JNK was almost completely suppressed by NAC (Fig. 6). This result confirms that TRAF2 was necessary to activate JNK after ER stress, and suggests that the increased level of ROS detected in the absence of TRAF2 may mediate the sustained activation of JNK. This is in agreement with previous reports showing that after TNF stimulation the early activation of JNK depends on TRAF2 and that the sustained activation of JNK depends on ROS (26). Altogether these results suggested that following ER stress, the TRAF2-mediated activation of NF-κB was responsible for protection from apoptosis by decreasing ROS levels and controlling sustained JNK activation.

DISCUSSION

The endoplasmic reticulum is the principal site for protein synthesis and folding, and also serves as a cellular storage site for calcium. Agents that interfere with protein folding or export lead to ER stress and eventually cell death. Although initiation of apoptosis induced by death receptors and mitochondria is well studied, the mechanism by which ER stress triggers apoptosis is still not clear. In the present paper, we present evidence supporting a central role played by TRAF2 in regulation of pro-apoptotic and anti-apoptotic pathways initiated at the ER. We demonstrate that TRAF22/− MEFs have increased susceptibility to ER stress-induced apoptosis. This increased susceptibility to ER stress-induced apoptosis was because of accumulation of ROS following ER stress, and was abolished by the use of antioxidants, such as NAC. In addition, we demonstrated that NF-κB was protecting cells from ER stress-induced apoptosis by controlling ROS accumulation.

TRAF2 has been demonstrated to be involved in signaling from endoplasmic reticulum being able to interact with Ire1 (10), one of the ER transmembrane proteins involved in initiating signals from the ER. TRAF2 mediates activation of both the JNK/SAPK and the NF-κB pathways following ER stress (10, 11). This scenario is reminiscent of TNF signaling, in which TRAF2 mediates simultaneous activation of the NF-κB survival pathway and pro-apoptotic JNK pathway, and the fate of the cell would be determined by interplay between these opposing signals. NF-κB exerts its anti-apoptotic activity by inhibiting caspase function (28–30), preserving function of mitochondria (31), and down-regulating JNK activity (23, 32). The latter function is mediated by at least two different mechanisms: by blocking activation of MKK7 via GADD45 (16) and decreasing ROS accumulation via the ferritin heavy chain (17). The importance of ROS in regulating sustained activation of JNK following TNF receptor triggering has been recently investigated in a NF-κB null cell model (26). Based on this study, TRAF2-mediated NF-κB activation suppresses the TNF-induced ROS accumulation that, in turn, induces prolonged JNK activation and cell death. Our result supports this model and suggests that a similar mechanism may also operate for the ER. In fact, induction of ER stress causes activation of both NF-κB and JNK. In the absence of TRAF2 or p65, the NF-κB...
pathway is not activated, and the late, ROS-dependent JNK activity is not counteracted, leading to cell death.

How does ROS affect JNK activation? ROS may affect JNK activation by at least two different mechanisms: by oxidizing and inhibiting mitogen-activated protein kinase phosphatase (33) and activating the protein ASK1 (34). This kinase may be activated via ROS and TRAF2 and has been demonstrated to be essential for inducing cell death after ER stress, at least in neuronal cells (34, 35). It may be possible that after ER stress and in the absence of a functional NF-κB activation, ASK1 is activated by the increased level of ROS and mediates sustained JNK activation and cell death.

Whereas it is clear from our results that the presence of a functional NF-κB is necessary for survival, counteracting increased induction of ROS following ER stress, the mechanism by which NF-κB exerts this function is not fully understood. It has been recently demonstrated that NF-κB up-regulates expression of ferritin heavy chain, an enzyme involved in iron metabolism and suppression of ROS accumulation (17). However, it is possible that in addition to up-regulation of genes involved in disposal of the ROS, NF-κB may also control transcription of genes that suppress production of ROS.

Our results confirm the central role played by TRAF2 in regulating activation of NF-κB following ER stress, and also sheds light on the functional significance of TRAF2-mediated NF-κB activation, another mechanism leading to activation of NF-κB following increased stress might exist. Based on this model, following ER stress, phosphorylation of eukaryotic initiation factor 2 represses synthesis of the inhibitory subunit IκBα, leading to activation of NF-κB (36). The two models of activation of NF-κB following ER stress, the TRAF2-mediated and the eukaryotic initiation factor 2-mediated, are not mutually exclusive. It is possible that both mechanisms contribute to activate NF-κB upon ER stress. However, whereas the biological significance of the link between eukaryotic initiation factor 2 phosphorylation and NF-κB activation is not fully understood, the functional significance of TRAF2-mediated NF-κB activation seems to be clear, at least in our experimental system. In fact, cells lacking TRAF2 or functional NF-κB undergo massive cell death after ER stress.

In conclusion, in the present study we provide evidence, for the first time, that the adaptor protein TRAF2 plays a central role in regulating signaling from the ER and that the activation of NF-κB, mediated by TRAF2, proteccts cells from ER stress-induced apoptosis. Therefore TRAF2 and NF-κB may be potential targets to control ER stress-induced apoptosis.

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REFERENCES
1. Berridge, M. J. (1995) Biochem. J. 312, 1–11
2. Sambrook, J. F. (1990) Cell 61, 197–199
3. Kaufman, R. J. (1999) Genes Dev. 13, 1211–1233
4. Sitia, R., and Braakman, I. (2003) Nature 426, 891–894
5. Tirasophon, W., Welshinda, A. A., and Kaufman, R. J. (1998) Genes Dev. 12, 1812–1824
6. Wang, X. Z., Harding, H. P., Zhang, Y., Jolicoeur, E. M., Kuroda, M., and Ron, D. (1998) EMBO J. 17, 5708–5717
7. Shi, Y., Vattem, K. M., Sood, R., An, J., Liang, J., Stramrn, L., and Wek, R. C. (1998) Mol. Cell. Biol. 18, 7499–7509
8. Bertolotti, A., Zhang, Y., Hendershot, L. M., Harding, H. P., and Ron, D. (2000) Nat. Cell Biol. 2, 326–332
9. Breckenridge, D. G., Germain, M., Mathai, J. P., Nguyen, M., and Shore, G. C. (2003) Oncogene 22, 8608–8618
10. Urano, F., Wang, X. Z., Bertolotti, A., Zhang, Y., Chung, P., Harding, H. P., and Ron, D. (2000) Science 287, 664–666
11. Leonard, A., Vito, P., Mauro, C., Pacifico, F., Ulianich, L., Consiglio, E., Formisano, S., and Di Jeso, B. (2002) Endocrinology 143, 2169–2177
12. Chung, J. Y., Park, Y. C., Ye, H., and Wu, H. (2002) J. Cell Sci. 115, 679–688
13. Rothe, M., Wong, S. C., Henzel, W. J., and Goeddel, D. V. (1994) Cell 78, 681–692
14. Yeh, W. C., Shaahiania, A., Speiser, D., Krausn, J., Billia, F., Wakeham, A., de la Pompa, J. L., Ferrick, D., Hurn, B., Isco, V., Ohashi, P., Rothe, M., Goeddel, D. V., and Mak, T. W. (1997) Immunity 7, 715–725
15. Lee, S. Y., Rechlin, A., Santana, A., Sokol, K. A., Nussenzweig, M. C., and Choi, Y. (1997) Immunity 7, 703–713
16. Papa, S., Zazzeroni, F., Rubici, C., Jayawardena, S., Alvarez, K., Matsuda, S., Nguyen, D. U., Pharm, C. G., Nelsbach, A. H., Melis, T., De Smaele, E., Tang, W. J., D’Adamio, L., and Fransozo, G. (2004) Nat. Cell Biol. 6, 146–153
17. Pham, C. G., Rubici, C., Zazzeroni, F., Papa, S., Jones, J., Alvarez, K., Jayawardena, S., De Smaele, E., Corng, R., Beauumont, C., Torti, F. M., Torti, S. V., and Fransozo, G. (2004) Cell 119, 529–542
18. Bray, T. M. (1999) Proc. Soc. Exp. Biol. Med. 222, 195
19. Forssberg, L., deFaire, U., and Morgenstern, R. (2001) Arch. Biochem. Biophys. 389, 84–93
20. Finkel, T. (2003) Curr. Opin. Cell Biol. 15, 247–254
21. Haynes, C. M., Titus, E. A., and Cooper, A. A. (2004) Mol. Cell 15, 767–776
22. Tournaire, C., Hess, F., Yang, D. D., Xu, J., Turner, T. K., Nimmul, A., Bar-Sagi, D., Jones, S. N., Flavell, R. A., and Davis, R. J. (2000) Science 288, 870–874
23. De Smaele, E., Zazzeroni, F., Papa, S., Nguyen, D. U., Jin, R., Jones, J., Cong, R., and Fransozo, G. (2001) Nature 414, 308–313
24. Leonard, A., E ligning-Ziegelbauer, H., Fransozo, G., Brown, K., and Siebenlist, U. (2000) J. Biol. Chem. 275, 271–278
25. Nicotelli, I., Miglioretti, G., Pagliacci, M. C., Grignani, F., and Riccardi, C. (1991) J. Immunol. Methods 139, 271–279
26. Sakon, S., Xue, Y., Takekawa, M., Sasazki, T., Okazaki, T., Kojima, Y., Piao, J. H., Yagita, H., Okumura, K., Doi, T., and Nakano, H. (2003) EMBO J. 22, 3898–3909
27. Deleted in proof
28. Deveraux, Q. L., Roy, N., Stennicke, H. R., Van Arsdale, T., Zhou, Q., Srinivasula, S. M., Alnemri, E. S., Salvesen, G. S., and Reed, J. C. (1998) EMBO J. 17, 2215–2223
29. Liston, P., Roy, N., Tamai, K., Lefebvre, C., Baird, S., Cherton-Horvat, G., Farahani, R., McLean, M., Ikeda, J. E., MacKenzie, A., and Korneluk, R. G. (1996) EMBO J. 15, 349–355
30. Muzio, M., Chinnaiyan, A. M., Kissik, F. C., O’Rourke, K., Scheden, A., Ji, N., Scaffidi, C., Breit, J. D., Zhang, M., Gentz, R., Mann, K., Krammer, P. H., Peter, M. E., and Dellit, V. M. (1996) Cell 85, 817–827
31. Boise, L. H., Gonzalez-Garcia, M., Posterna, C. E., Ding, L., Lindsten, T., Turk, L. A., Mao, X., Nunez, G., and Thompson, C. B. (1993) Cell 74, 597–608
32. Tang, G., Minemoto, Y., Dilling, B., Purcell, N. H., Li, Z., Karin, M., and Lin, A. (2001) Nature 414, 313–317
33. Kamata, H., Honda, S., Maeda, S., Chang, L., Hirata, H., and Karin, M. (2005) Cell 120, 649–661
34. Totienu, K., Matsuzawa, A., Takahashi, T., Nishihori, H., Morita, K., Takeda, K., Ninora, O., Miyazono, K., Noda, T., and Ichijo, H. (2001) EMBO J. 20, 222–228
35. Nishihori, H., Matsuzawa, A., Totienu, K., Saegusa, K., Takeda, K., Inoue, K., Kakizuka, A., and Ichijo, H. (2002) Genes Dev. 16, 1345–1355
36. Deng, J., Lu, D. L., Zhang, Y., Scheweuer, D., Kaufman, R. J., Sonenberg, N., Harding, H. P., and Ron, D. (2004) Mol. Cell. Biol. 24, 10161–10168