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Dual function of human tumor necrosis factor receptor 75 in cytotoxicity induced by human tumor necrosis factor α

FANG Jia, LU Fang, CHEN Chang-Qing[1]
(*Shanghai Research Center of Biotechnology, Chinese Academy of Sciences, Shanghai 200233, China*)

KEY WORDS tumor necrosis factor; site-directed mutagenesis; tumor necrosis factor receptors; gene expression; cell survival; protein binding; signal transduction

ABSTRACT

AIM: To study the function of human TNF receptor-75 (hTR75) and the interaction between human TNF receptor-55 (hTR55) and hTR75 in hTNFα-induced cytotoxicity. **METHODS**: HEp-2 cells were transfected with bicistronic expression vector of hTR75 gene, and HEp-2-A75 cells with intrinsic hTR55 and overexpressed hTR75 were obtained. Two hTNFα muteins with exclusive specificity for hTR55 or hTR75 were constructed, expressed in high-levels in *E coli*, and then purified. hTNFα-induced cytotoxicity was determined by crystal violet colorimetric method. **RESULTS**: The expression of hTR75 in HEp-2 cells was demonstrated by RT-PCR and indirect ELISA, and was quantified by binding of [^{125}I] hTNFα and Scatchard analysis. The overexpressed hTR75 could markedly increase the susceptibility of HEp-2 cells to hTNFα. **CONCLUSION**: hTR75 could not only partially mediate hTNFα-induced cytotoxicity independently but also fulfill an accessory role in enhancing or synergizing hTR55-mediated cytotoxicity. It played a dual function in hTNFα-induced cytotoxicity in HEp-2 cells.

INTRODUCTION

Tumor necrosis factor alpha (TNFα), produced mainly by activated macrophages, is one of the most pleiotropic cytokines[1]. The first step in TNF action is binding to specific receptors that are expressed on virtually all nucleated cells. Two distinct receptors for TNF have been identified, TNF receptor 55 (TR55, M_r = 55000) and TNF receptor 75 (TR75, M_r = 75000)[2]. Both receptors belong to TNF receptor superfamily because they share 28 % homology and contain 4 conserved cysteine-rich subdomain in their extracellular regions[3]. It is the unique structural features of receptors that allow them to recognize TNF with specificity. The ligand binding properties of TR55 and TR75 have been extensively studied. In a recent report the rapid kinetics of TR75 association and dissociation have been taken as a basis to postulate a model termed "ligand passing" in which the ligand bound to TR75 may be passed over to TR55 to enhance TR55 signaling[4].

The intracellular domains of the two TNF receptors have no sequence homology, suggesting distinct biological functions[2]. Deletion mutagenesis of TR55 revealed that a death domain[5], which consists of about 80 amino acids residues located to the C-terminal portion of the protein's intracellular region, is responsible for cytotoxicity and NF-κB activation. Intracellular region of TR75 does not possess the death domain or any other domains with intrinsic catalytic activity. An adapter protein, TRAF2 (TNFR-associated factor-2), was found to function as the actual signal transducer in TR75-mediated signaling[6]. It has been shown recently that the TR55-TRADD (TNFR-associated death domain) complex could also bind to RIP (receptor-interacting protein) and then interact with TRAF2[7]. Thus, the ligand-induced interaction between TR55 and TR75 may have important implications in TNF signaling. In the present study we investigated the function of human TR75 (hTR75) in hTNFα-induced cytotoxicity in HEp-2 cells.

MATERIALS AND METHODS

Reagents and kits Restriction endonucleases, AMV reverse transcriptase, *Taq* DNA polymerase, dNTP, and RNasin were from Roche or Promega; Geneticin (G-418), TRIzol reagent, trypsin, and

[1] Correspondence to Prof CHEN Chang-Qing.
Phn 86-21-6470-0892, ext 306. Fax 86-21-6470-0244.
E-mail cqchen@srcb.ac.cn
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Dulbecco's modified Eagle's medium (DMEM) were from Gibco BRL; newborn bovine serum (NCS) was from Hangzhou Sijiqin Biotechnology Company; McAbs to hTR55 or hTR75 were from R&D Systems, UK; HRP-labeled goat anti mouse IgG, cycloheximide (CHX) were from Sigma; Standard MW markers of protein and nucleic acid were from Sino-American Biotechnology Company; [^{125}I]NaI was a product of Amersham; Wild-type hTNFα was expressed in *E coli* and purified in our lab; hTNFα was labeled with ^{125}I to specific radioactivity of 1.15 – 1.76 TBq/g.

Bacterial, plasmid, and cell line *E coli* BL-21 [*E coli* BF$^-$, *omp*T, *hsd*S(r_B^-, m_B^-), *gal*, *dcm*$^-$, wt-hTNFα and R32W-L157F-hTNFα genes, and *E coli* expression vector pSB92 were from Gene Bank of Chinese Academy of Sciences; human larynx carcinoma-derived cell line, HEp-2 (Aarhus University, Denmark) was cultured in DMEM containing 10 % NCS at 37 ℃ in 95 % air + 5 % CO_2.

Generation of HEp-2 cells expressing hTR75 The bicistronic expression vector[8], containing hTR75 gene and *neo*^{R} gene, was introduced into HEp-2 cells by electroporation. Cells (5×10^6 in 1.0 mL) were transfected with 10 μg of pA75 plasmid (376 V, 1080 μF). The next day, G418 was added to a final concentration of 800 g/L. About two weeks later, individual colonies were picked up and expanded. To examine the expression of hTR75, cells (4×10^4/well) were seeded into 96-well microtiter plates in 100 μL of medium and incubated overnight. The cells were then fixed by 0.25 % glutaraldehyde and expression of hTR75 was detected by McAbs to hTR75 and anti-mouse IgG-HRP. Expression of hTR75 was also monitored by RT-PCR. RNA was prepared by TRIzol reagent from 2×10^6 cells. After reverse transcription reaction, PCR was carried out with sense primer (CCGCCCAGGTGG-CATTTAC) and antisense primer (ATACTCGAGTGC-CCCTGGGGCCA).

Receptor binding assay[9] Cells were trypsinized and seeded into 24-well plates at 2.5×10^6 cells/well. After incubation overnight at 37 ℃, 5 % CO_2, the culture medium was aspirated and increasing concentrations of [^{125}I]hTNFα in 500 μL binding buffer (PBS containing 0.2 % BSA and 0.02 % sodium azide) were added to triplicate wells. After incubation at 4 ℃ for 2 h, the cell monolayers were washed thrice with binding buffer, solubilized in PBS containing 2 mol/L NaOH and 50 mmol/L edetic acid, then the radioactivity was determined in γ-counter (Beckman 5500B). Specific binding was calculated as the difference between the cell-associated counts in the absence and presence of a 500-fold excess of unlabeled ligand.

Preparation of two human TNFα muteins Site-directed mutation by overlap-PCR was carried out as described[10]. The mutant R32W-S86T-hTNFα was constructed using P2 and P3 primers, R32W-L157F-hTNFα gene as the template. The mutant D143F-hTNFα was constructed using P4, P5, and P6 primers, wild-type hTNFα gene as its template. Then the complete mutant genes were amplified by using P1 and P7 primers. P1 (plus): 5′-GATACGAAACGAAGCATTGGTTAA-3′; P2 (plus): 5′-GTATTGCTGTTACCTACCAGAC-3′; P3 (minus): 5′-GTCTGGTAGGTAACAGCAATAC-3′; P4 (plus): 5′-CCAGACTACCTTTTCTTCGCTGAATCTG-3′; P5 (minus): 5′-GATTCAGCGAAGAAAAGGTA-GTCTGGAC-3′; P6 (in pSB92, minus): 5′-TGTCCTACTCAGGAGAGCGT-3′; P7 (minus): 5′-T-GTGGATCCTCACAGAGCGATAATACC-3′. Methods for expression and purification of the hTNFα muteins have been described previously[11].

Competitive binding activity of hTNFα with receptors Wild-type hTNFα was coated in 96-well microtiter plates at 0.2 – 1 μg/well in 100 μL carbonate buffer (pH 9.6) and incubated overnight at 4 ℃. After washing and blocking, hTNFα muteins R32W-S86T-hTNFα or D143F-hTNFα were diluted serially in medium containing hsTR55-preS1 or hsTR75-preS1 fusion receptors, and added to the well. After incubation for 1 – 2 h at 37 ℃, HRP labeled anti-HBsAg preS1 McAbs were added. TMB was used as substrate, and optic density was measured at 570 nm.

Cytotoxicity assay HEp-2 or HEp-2-A75 cells were seeded into 96-well microtiter plates at 4×10^4/well in 100 μL medium and allowed to grow for 22 – 24 h. Cycloheximide was added to a final concentration of 20 mg/L, and wild-type hTNFα or its muteins were added to the wells in serial dilution. The plates were incubated at 37 ℃. At different time points, the viable cells were stained with staining buffer (22.3 % ethanol containing 0.5 % crystal violet, 8 % methanol, 7 g/L NaCl) for 1 – 2 h. The dye was eluted with 33 % citric acid, and absorbance was measured at 595 nm.

RESULTS

Construction of two hTNFα mutant genes and their expression vectors The PCR products of

mutated genes were digested with *Bam*H Ⅰ and *Eco*R Ⅰ and cloned into *E coli* expression vector pSB92, which contains P_L promoter and clts857. All of the constructs were identified with restriction endonucleases digestion and sequence analysis.

Expression, purification, and characterization of recombinant hTNFα muteins SDS-PAGE analysis of total cell lysates revealed that, after 4-h expression at 42 ℃, two hTNFα muteins were expressed at levels of 40 % – 50 % of total cellular proteins. The muteins, which were partially soluble, were precipitated from supernatant after sonication by ammonia sulfate and further purified by ion-exchange chromatography. The purity was over 90 % (Fig 1). Competitive binding experiments demonstrated that R32W-S86T-hTNFα and D143F-hTNFα had the expected exclusive specificity for the hTR55 or hTR75, respectively (Tab 1).

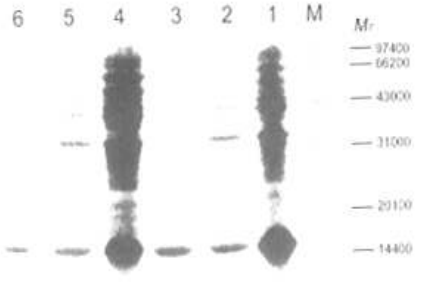


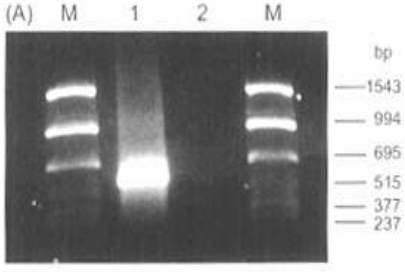
Fig 1. SDS-PAGE analysis of R32W-S86T-hTNFα and D143F-hTNFα. M) Molecular weight marker of protein; 1) Expression of R32W-S86T-hTNFα; 2) Supernatant of R32W-S86T-hTNFα after sonication; 3) Purified products of R32W-S86T-hTNFα; 4) Expression of D-143F-hTNFα; 5) Supernatant of E143F-hTNFα after sonication ; 6) Purified products of D143F-hTNFα.

Tab 1. Competitive binding assays of hTNFα and its mutants to hTR55 and hTR75.

Mutants	Relative binding ability/ %	
	hTR55	hTR75
Wt-hTNFα	100	100
R32W-S86T-hTNFα	8.9	< 0.26
D143F-hTNFα	< 0.098	5.5

Relative binding ability was expressed as the ratio of mutants to wt-hTNFα.

Expression of hTR75 in HEp-2 cells The expression of hTR75 in HEp-2 cells at transcription and translation levels was analyzed by RT-PCR (Fig 3A) and indirect ELISA (Fig 2B), respectively. The number of receptors in HEp-2-A75 cells was measured by [^{125}I]hTNFα binding experiments and derived from Scatchard plots. Analyses reveal that HEp-2-A75 cells possess about 9.75×10^5 binding sites per cell and a K_d of 4.06 nmol/L (Fig 3).



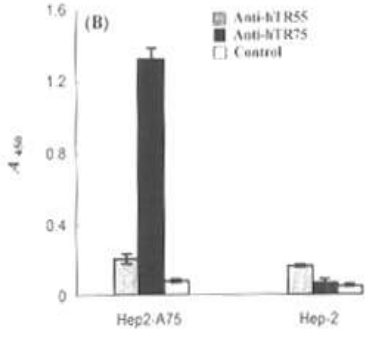


Fig 2. Identification of Hep-2 cells transfected by hTNFR (A) RT-PCR analysis; (B) Indirect ELISA. M) PCR marker; 1) hTR75 RT-PCR of HEp-2-A75 cells; 2) hTR75 RT-PCR of HEp-2 cells. $n = 3$. $x \pm s$.

Cytotoxicity assay of HEp-2 and HEp-2-A75 cells In the presence of cycloheximide, wild-type hTNFα and R32W-S86T-hTNFα appeared obviously cytotoxic to HEp-2 and HEp-2-A75 cells. For HEp-2 cells, R32W-S86T-hTNFα with EC_{50} 13.3 μg/L showed lower cytotoxicity compared with wild-type hTNFα (EC_{50} was 2.37 μg/L), and D143F-hTNFα was not cytotoxic (Fig 4A). Compared with HEp-2 cells, HEp-2-A75 cells increased their sensitivity to wild-type hTNFα more than 1×10^5 folds and to R32W-S86T-hTNFα (EC_{50} 0.1 μg/L) about 100 folds after being treated for 12 h together with cycloheximide. Furthermore, cytotoxicity by D143F-hTNFα was also observed in high concentration and its EC_{50} was 316 μg/L (Fig 4B).

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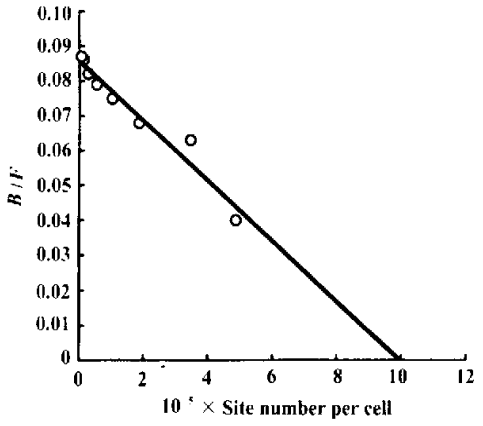

Fig 3. Scatchard analysis of [^{125}I]hTNFα to HEp-2-A75 cells.

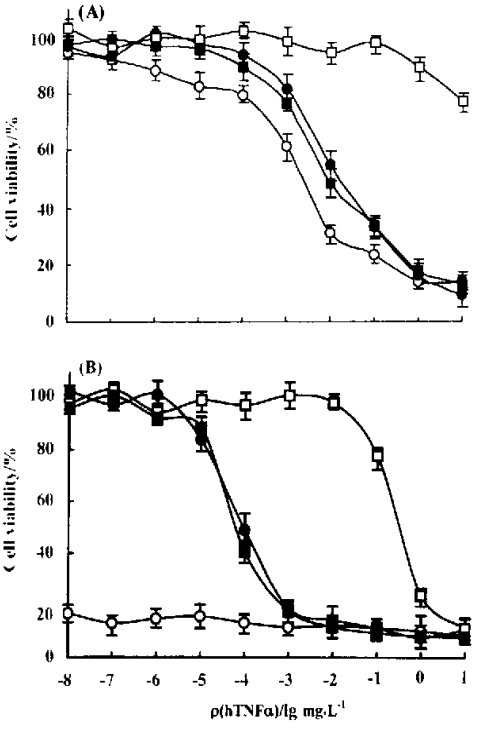

Fig 4. Cytotoxic effect of hTNFα on HEp-2 and HEp-2-A75 cells (12 h). (A) HEp-2; (B) HEp-2-A75. (○) wt-hTNFα; (●) R32W-S86T-hTNFα; (_) D143F-hTNFα; (■) R32W-S86T-hTNFα + D143F-hTNFα. $n=3$. $\bar{x} \pm s$.

We also noticed the difference in susceptibility to hTNFα between HEp-2 cells and HEp-2-A75 cells. For the latter, cell death could be observed after 3 h treatment with hTNFα 1 μg/L. However, under the same conditions, 8-h treatment was required before HEp-2 cells showed sensitivity to hTNFα. In addition, the process of hTNFα-induced cytotoxicity in HEp-2 cells was slower than in HEp-2-A75 cells (Fig 5A, 5B). These observations implicated that the hTR75 played a dual function in hTNFα-induced cytotoxicity in HEp-2 cells.

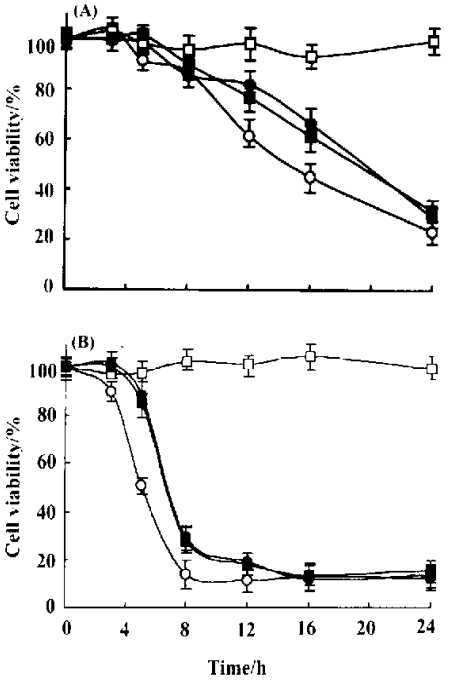

Fig 5. Time course of cytotoxic effect of hTNFα and it's muteins (1 μg/L) on HEp-2 (A) and HEp-2-A75 cells (B). (○) wt-hTNFα; (●) R32W-S86T-hTNFα; (□) D143F-hTNFα; (■) R32W-S86T-hTNFα + D143F-hTNFα. $n=3$. $\bar{x} \pm s$.

DISCUSSION

Both TNF receptors, TR55 and TR75, are expressed in the majority of cell types and tissues. However, it is established that TR55 is necessary and sufficient for TNFα-induced cellular responses[12] through interacting with several adapter proteins, such as TRADD, FADD, RIP, *etc*. In contrast, TR75 appears to mediate only a limited number of cellular responses[13]. Despite the fact that the adapter protein for TR75, TRAF-2[6] has been identified several years ago, little is known about the accurate mechanism of signal pathways utilized by TR75.

HEp-2, a human larynx carcinoma-derived cell line,

only expresses human TR55 and is susceptible to the cytotoxic action of hTNFα in the presence of cycloheximide[14]. This made it an ideal model system for studying hTNFα signal pathways. In the present study, we introduced the biocistronic vector of hTR75 and *neo*^{R} genes into HEp-2 cells by electroporation. The transcription of both genes was controlled by LTR promoter while the translation of hTR75 and *neo*^{R} was controlled by 5′-cap structure and EMCV IRES (internal ribosome entry site)[15], respectively. The usage of the bicistronic vector increases the translation efficiency of interested gene and simplifies the following screening of transfects[16]. Furthermore, we demonstrated that EMCV IRES was functional in HEp-2 cells though it is host selective.

Structure-function studies of hTNFα showed that Asp143 was the key residue for binding to hTR55 while Arg32 and Ser86 to hTR75[17]. In order to understand the accurate cellular responses mediated by hTR55 or hTR75, we constructed the hTR55-specific mutant R32W-S86T-hTNFα and the hTR75-specific mutant D143F-hTNFα with overlapping PCR. Both muteins were expressed in *E coli* at high levels, and target proteins were purified. Their exclusive specificity for hTR55 or hTR75 was confirmed by the receptor competitive binding assay.

The cytotoxicity induced by wild-type hTNFα and its muteins in HEp-2 and Hep-2-A75 cells was further compared. We found that overexpressed hTR75 could not only independently mediate cytotoxicity, but also play an accessory role in enhancing or synergizing hTR55-mediated cytotoxicity. However, the cytocidal effect curve of HEp-2-A75 cells shows that the hTR75-mediated cytotoxicity requires high concentrations of both ligand and receptor. It is obviously different from that mediated by hTR55. The exact mechanism of this phenomenon is far from well understood. It is possible that hTR75 clustering after binding to hTNFα is far weaker than hTR55 clustering because the intracellular domain of hTR75 lacks the death domain, which has a strong tendency to self-associate[18]. Furthermore, Grell *et al*[19] recently reported that the affinity of binding to hTNFα and the stability of hTNFα-receptor complex for hTR55 were remarkably higher than those for hTR75 under physiological conditions (37 ℃). We think the difference in ligand-receptor binding characteristics between hTR55 and hTR75 may also explain the differential behavior of the two receptors.

Our data also shows the interaction between hTR55 and hTR75 in hTNFα-induced cytotoxicity. On the one hand, the ligand passing model could explain why HEp-2-A75 cells were more susceptible by far to the cytotoxic action of wild-type hTNFα than its muteins (Fig 5). On the other hand, we also found that HEp-2-A75 cells increased their susceptibility to R32W-S86T-hTNFα about 100 folds compared with HEp-2 cells. It is possible that unliganded hTR75 could also synergize hTR55-mediated cytotoxicity through its intracellular domain.

Now, many signaling molecules have been identified, such as TRADD, FADD, TRAFs and so on[14]. TRAF2, a TR75 binding protein, could associate with the C-terminal 78 amino acid region of TR75 in TRAF2 homodimers or TRAF1/TRAF2 heteromeric complex and then activate signal pathways. Meanwhile, clustered TR55 could bind its adapter protein TRADD through interaction between their death domains. The death domain of TRADD could also associate with the corresponding domain of FADD. Activation of the later could further activate the caspase cascade and lead to apoptosis[20]. Hsu *et al*[21] reported that TRADD could also interact with TRAF2 and RIP, and thus stimulate pathways leading to activation of NF-κB and JUK/AP-1. Therefore, TRAF2 might play a key role in TR55 and TR75 interaction. However, there are reports that dominant negative mutation of TRAF2 was not able to affects receptor cooperation[22]. Thus when the above and related questions are further clarified, our knowledge of TNF function would be more clear.

REFERENCES

1 Beutler B, Cerami A. Tumor necrosis, cachexia, shock and inflammation: a common mediator. Ann Rev Biochem 1988; 57: 505 – 18.

2 Vandenabeele P, Declercq W, Beyaert R, Fiers W. Two tumour necrosis factor receptors: structure and function. Trends Cell Biol 1995; 5: 392 – 9.

3 Beutler B, van Huffel C. Unraveling function in the TNF ligand and receptor families. Science 1994; 264: 667 – 8.

4 Tartaglia LA, Pennica D, Goeddel DV. Ligand passing: the 75-kD tumor necrosis factor (TNF) receptor recruits TNF for signaling by the 55-kDa TNF receptor. J Biol Chem 1993; 268: 18542 – 8.

5 Tartaglia LA, Ayres TM, Wong GH, Goeddel DV. A novel domain within the 55 kD TNF receptor signals cell death. Cell 1993; 74: 845 – 53.

6 Rothe M, Wong SC, Henzel WJ, Goeddel DV. A novel family of putative signal transducers associated with the cytoplasmic domain of the 75 kDa tumor necrosis factor receptor. Cell 1994; 78: 681 – 92.

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E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

7 Kelliher MA, Grimm S, Ishida Y, Kuo F, Stanger BZ, Leder P. The death domain kinase RIP mediates the TNF-induced NF-kappaB signal. Immunity 1998; 8: 297 – 303.
8 Fang J, Zhu B, Wang DB, Chen CQ, Duch MR, Pedersen FS. Overexpressed human TNF receptor-75 can independently mediate hTNFα-induced cytotoxicity in BHK-21 cells. Acta Biochim Biophys Sin 1999; 31: 637 – 42.
9 Carlino JA, Lin LS, Creasey AA. Use of a sensitive receptor binding assay to discriminate between full-length and truncated human recombinant tumor necrosis factor proteins. J Biol Chem 1987; 262: 958 – 61.
10 Higuchi R, Krummel B, Saiki RK. A general method of *in vitro* preparation and specific mutagenesis of DNA fragments: study of protein and DNA interaction. Nucleic Acids Res 1988; 16: 7351 – 67.
11 Xu H, Gao CS, Liu H, Deng F, Pan H, Chen CQ. Downstream technique study of human tumor necrosis factor alpha and its mutant. Chin J Biotechnol 1998; 14: 166 – 71.
12 Tartaglia LA, Goeddel DV. Two TNF receptors. Immunol Today 1992; 13: 151 – 3.
13 Baker SJ, Reddy EP. Modulation of life and death by the TNF receptor superfamily. Oncogene 1998; 17: 3261 – 70.
14 Brockhaus M, Schoenfeld HJ, Schlaeger EJ, Hunziker W, Lesslauer W, Loetscher H. Identification of two types of tumor necrosis factor receptors on human cell lines by monoclonal antibodies. Proc Natl Acad Sci USA 1990; 87: 3127 – 31.
15 Borman AM, Mercier PL, Girard M, Kean KM. Comparison of picornaviral IRES-driven internal initiation of translation in cultured cells of different origins. Nucleic Acid Res 1997; 25: 925 – 32.
16 Belsham GJ, Sonenberg N. RNA-protein interaction in regulation of picornavirus RNA translation. Microbiol Rev 1996; 60: 499 – 514.
17 Loetscher H, Stueber D, Banner D, Mackay F, Lesslauer W. Human tumor necrosis factor α (TNFα) mutants with exclusive specificity for the 55-kDa or 75-kDa TNF receptors. J Biol Chem 1993; 268: 26350 – 7.
18 Haridas V, Darnay BG, Natarajan K, Heller R, Aggarwal BB. Overexpression of the p80 TNF receptor leads to TNF-dependent apoptosis, nuclear factor-κB activation, and c-*Jun* kinase activation. J Immunol 1998; 160: 3152 – 62.
19 Grell M, Wajant H, Zimmermann G, Scheurich P. The type 1 receptor (CD120a) is the high-affinity receptor for soluble tumor necrosis factor. Proc Natl Acad Sci USA 1998; 95: 570 – 5.
20 Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. Science 1998; 281: 1305 – 8.
21 Hsu H, Shu HB, Pan MG, Goeddel DV. TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal transduction pathways. Cell 1996; 84: 299 – 308.
22 Declercq W, Denecker G, Fiers W, Vandenabeele P. Cooperation of both TNF receptors in inducing apoptosis: involvement of the TNF receptor-associated factor binding domain of the TNF receptor 75. J Immunol 1998; 161: 390 – 9.

人肿瘤坏死因子受体 75 在人肿瘤坏死因子 α 介导的细胞毒效应中的双重功能

方 佳，卢 芳，陈常庆[1]（中国科学院上海生物工程研究中心，上海 200233，中国）

关键词 肿瘤坏死因子；定点诱变；肿瘤坏死因子受体；基因表达；细胞存活；蛋白质结合；信号传递

目的：研究人肿瘤坏死因子受体 75（hTR75）在 hTNFα 所引发的细胞毒效应中的功能. **方法**：用电脉冲将 hTR75 基因表达载体转入只有内源性 hTR55 表达的 HEp-2 细胞中构建了两种 TNF 受体同时表达的 HEp-2-A75 细胞株. 通过定点突变构建及在大肠杆菌中高效表达，分离纯化出两种分别对 hTR55 以及 hTR75 受体具有选择结合活性的 hTNFα 突变体. 野生型 hTNFα 及其这两种突变体对 HEp-2 以及 HEp-2-A75 细胞的细胞毒效应采用比色法测定并进行了比较. **结果**：RT-PCR 以及间接 ELISA 的结果表明 hTR75 在 RNA 转录以及蛋白质翻译水平均获得了表达，其表达量由 Scatchard 分析确定. HEp-2 细胞中 hTR75 的存在可以明显增强 HEp-2 细胞对 hTNFα 的敏感性. **结论**：hTR75 受体在介导 hTNFα 的细胞毒活性中具有双重功能，即 hTR75 不但自身可介导部份 hTNFα 所引发的细胞毒效应，它对 hTR55 所介导的细胞毒效应还具有相当的协同作用.

（责任编辑 朱倩蓉）