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World J Gastroenterol 2008 March 7; 14(9): 1353-1357
World Journal of Gastroenterology ISSN 1007-9327

BASIC RESEARCH

Expression of tumor necrosis factor-alpha converting enzyme in liver regeneration after partial hepatectomy

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Received: July 5, 2007 Revised: December 3, 2007

Abstract

AIM: To study the expression of tumor necrosis factor-alpha converting enzyme (TACE) and evaluate its significance in liver regeneration after partial hepatectomy *in vivo*.

METHODS: Male SD rats underwent 70% partial hepatectomy. The remaining liver and spleen tissue samples were collected at indicated time points after hepatectomy. TACE expression was investigated by Western blotting, immunohistochemistry, and serial section immunostaining.

RESULTS: Expression of TACE in liver and spleen tissues after partial hepatectomy was a time-dependent alteration, reaching a maximal level between 24 and 48 h and remaining elevated for more than 168 h. TACE protein was localized to mononuclear cells (MNC), which infiltrated the liver from the spleen after hepatectomy. The kinetics of TACE expression was in accordance with the number of TACE-staining MNCs and synchronized with those of transforming growth factor-α (TGFα). In addition, TACE-staining MNC partially overlapped with $CD3^+$ T lymphocytes.

CONCLUSION: TACE may be involved in liver regeneration by pathway mediated with TGFα-EGFR in the cell-cycle progressive phase *in vivo*. TACE production and effect by paracrine may be a pathway of involvement in liver regeneration for the activated $CD3^+$ T lymphocytes.

Key words: Tumor necrosis factor-alpha converting enzyme; Liver regeneration; Partial hepatectomy

Peer reviewer: Bruno Stieger, Professor, Department of Medicine, Division of Clinical Pharmacology and Toxicology, University Hospital, Zurich 8091, Switzerland

Lin XM, Liu YB, Zhou F, Wu YL, Chen L, Fang HQ. Expression of tumor necrosis factor-alpha converting enzyme in liver regeneration after partial hepatectomy. *World J Gastroenterol* 2008; 14(9): 1353-1357 Available from: URL: http://www.wjgnet.com/1007-9327/14/1353.asp DOI: http://dx.doi.org/10.3748/wjg.14.1353

INTRODUCTION

Tumor necrosis factor-alpha converting enzyme (TACE) is a kind of metalloprotease disintegrins, also known as ADAM17, which is a modular transmembrane protein with a zinc-dependent catalytic domain[1]. TACE was originally cloned and named for its ability to cleave and convert tumor necrosis factor-α (TNFα) into a soluble form. Since then, TACE has been demonstrated to solubilize a variety of substrates including transforming growth factor-α (TGFα), members of the membrane-bound epidermal growth factor (EGF) family ligands, both TNFR- I and TNFR-Ⅱ, and macrophage/colony-stimulating factor receptor[2]. Liver regeneration after partial hepatectomy is very intricate. The process requires the activation of more than 100 genes and involves multiple cytokines and growth factors such as interleukin- I , hepatocyte growth factor (HGF), TNFα, TGFα, heparin-binding epidermal growth factor-like growth factor (HB-EGF) *etc*[3,4]. It has been well documented that TACE mRNA and TACE protein are enhanced in several human malignant diseases such as breast cancer, lung cancer, and liver cancer[5,6]. In a study of hepatocyte replication, Gretchen *et al* employed the AML-12 hepatocyte cell line and implicated the participation of TACE in hepatocyte replication by activating the TGFα-EGFR pathway[7]. Hence, it is speculated that TACE is involved in cell proliferation and carcinogenesis in addition to inflammation[8-10]. It was also reported that the activity of several metalloproteinases increased during liver regeneration[11]. However, to date, there has been few studies on the correlation of TACE with liver regeneration after partial hepatectomy *in vivo*. To gain further insight into the involvement of TACE in liver regeneration, we investigated the expression of TACE in both liver and spleen tissues with a rodent partial hepatectomy model.

MATERIALS AND METHODS

Animals and study protocol

All animal experiments were performed following the

institution's criteria for the care and use of laboratory animals in Zhejiang University, China. Male SD rats 200-250 g) were fed standard rodent chow and water *ad libitum* in a temperature-controlled room. Rats were anesthetized with ether and underwent 70% partial hepatectomy according to the method of Higgins and Anderson. At indicated time points after hepatectomy, laparotomy was performed on the rats, and liver and spleen tissue samples were collected. A tissue sample was flash-frozen in liquid nitrogen for Western-blotting analysis, and the remainder was fixed in 4% formaldehyde and embedded in paraffin for immunohistochemical analysis. This study protocol was approved by the Ethics Committee of Zhejiang University, China.

Immunohistochemical analysis

Four μm-thick paraffin sections of liver and spleen tissue samples were cut. After deparaffinization, the endogenous peroxidase activity was blocked by placing the slides in methanol containing 3% (w/v) H_2O_2 for 30 min at room temperature. Normal goat serum was added and kept at room temperature for 15 min. The primary antibodies, rabbit anti-TACE polyclonal antibodies (1:100 dilution; CHEMICON) and mouse monoclonal anti-CD3 antibodies (1:200 dilution; Acris) were applied overnight at 4℃. After the slides were washed in phosphate buffered saline, the Envision + R system labelled polymer-HRP (Dako; Cytomation) was added and visualized using the DAB chromogen (Merck; Germany). Counterstaining of cell nuclei was accomplished with Mayer's hematoxylin (Sigma). Finally, sections were counterstained with hematoxylin, dehydrated, coverslipped, and evaluated microscopically. Positive-staining cells were counted for each visual field at 400 × magnification.

Western blot analysis

For total protein extraction, samples of rat liver and spleen tissues were homogenized in NETN buffer supplemented with protease inhibitors and centrifuged at 15000 r/min for 60 min at 4℃. Homogenates containing 50 μg of protein were loaded. The proteins were size-separated by electrophoresis on 7.5% polyacrylamide gels (Bio-Rad), and then transferred onto PVDF membranes (Bio-Rad). After blocking, membranes were incubated with a rabbit polyclonal antibody against rat TACE (1:1000 dilution; CHEMICON), and then with an alkaline phosphatase-conjugated anti-rabbit antibody (1:5000 dilution; Amersham). Immunoreactive proteins were detected with a fluorescence scanner (Storm, Pharmacia) using ECF substrate according to the manufacturer's instructions (Amersham). In control experiments, the membrane was incubated with a mouse monoclonal anti-β-actin antibody (1:1000 dilution; Sigma) and with an alkaline phosphatase-conjugated anti-mouse antibody (1:5000 dilution; Amersham).

RESULTS

TACE expression localization and pattern in liver and spleen following partial hepatectomy

To investigate TACE protein localization and pattern, we examined liver tissue paraffin sections by immunohistochemistry. Mononuclear cells (MNC) stained positively, but hepatocytes, biliary epithelia cells, and endothelial cells did not. There were few MNC in portal trials, and TACE staining could be hardly detected prior to hepatectomy; however, after hepatectomy, the TACE-staining MNC was observed to infiltrate to periportal sites. Marked accumulation of MNC was found at periportal sites from 24 to 48 h, while intense TACE-staining MNC was visible. The TACE-staining MNC became less abundant and distributed to the intermediate regions after 72 h (Figure 1). Because many TACE-staining MNCs were detected in the portal vein and it was speculated that the MNC mainly came from the spleen through portal vein current (Figure 2), we performed further immunohistochemical studies on spleen tissues. The kinetic feature of TACE-staining in spleen is analogous to that in liver, while the TACE-staining MNC reached the marginal zone and splenic sinus (Figure 3). The quantities of TACE-staining MNC at various time points in liver and spleen of 7 rats in each group were counted randomly under microscopy (Figure 4).

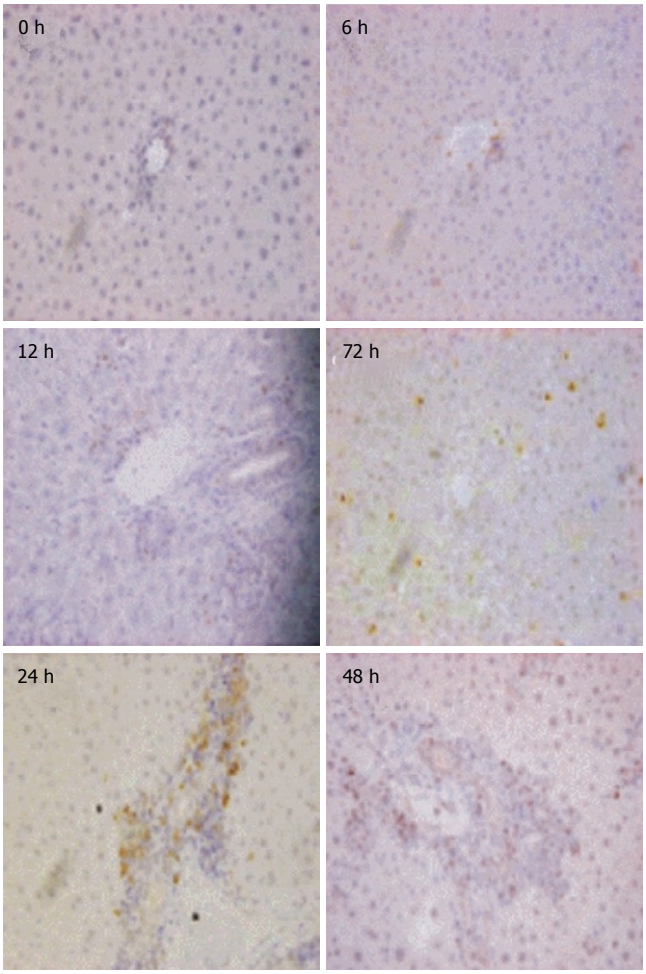

Figure 1 Rat liver stained using anti-TACE antibodies at various time points after hepatectomy. None of the parenchymal cells was stained at any of the time points. MNC accumulated markedly at periportal sites from 24 to 48 h after hepatectomy while intense TACE staining was seen. MNC declined and distributed to the intermediate regions after 72 h (× 400).

TACE expression level in liver and spleen following partial hepatectomy

The TACE expression levels in liver and spleen tissues at

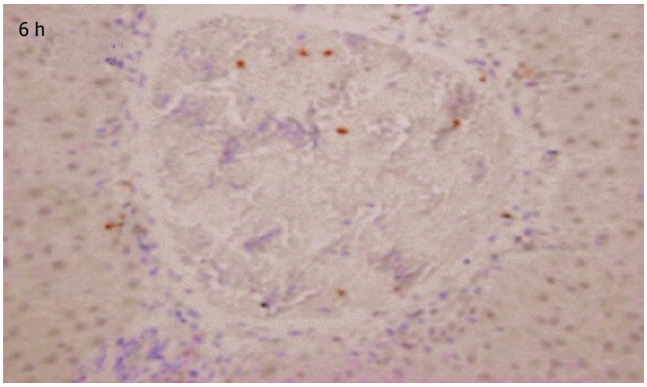

Figure 2 The TACE stained MNC in portal vein current 6 h after hepatectomy. The MNC infiltrated to periportal sites through portal triad vein endothelial cells (× 400).

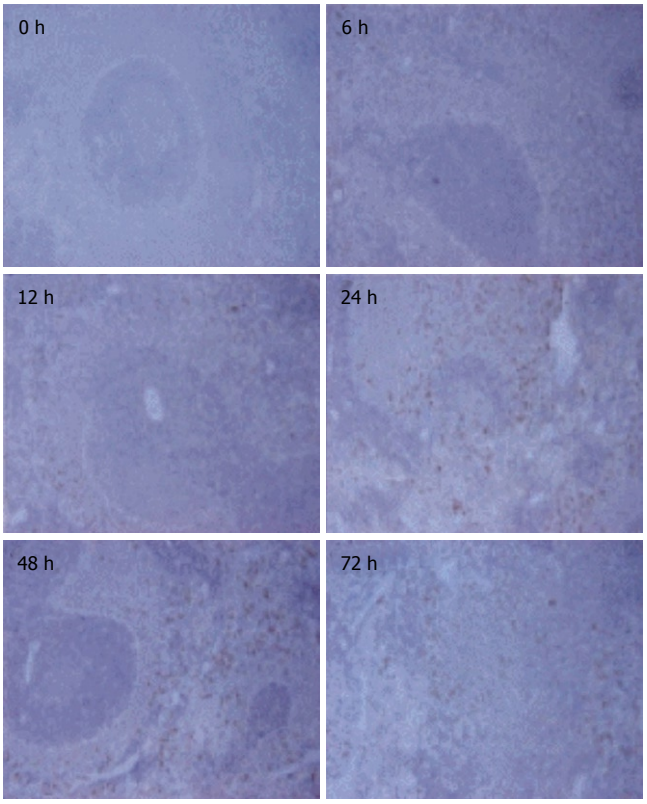

Figure 3 Immunostaining of TACE in rat spleen tissue at various time points. The TACE-stained MNC increased, reaching a peak at similar time points with liver. The positively stained MNC distributed at the marginal zone and splenic sinus. Numbers indicate the time points after hepatectomy (× 400).

various time points were evaluated by Western blotting. Because the antibody was directed against the cytoplasmic domain of the protein, both the precursor (pro-TACE) and the mature forms were detected. As shown in Figure 5, TACE expression in the liver and spleen after hepatectomy is a time-dependent alteration, reaching a maximal level between 24 h and 48 h. The TACE level declined from 72 h, but remained elevated for more than 168 h as compared with the pre-hepatectomy level in liver tissues.

Correlation of TACE-staining MNC with T lymphocytes

To identify the stained MNC, we immunostained serial paraffin sections of liver tissues at 48 h after hepatectomy

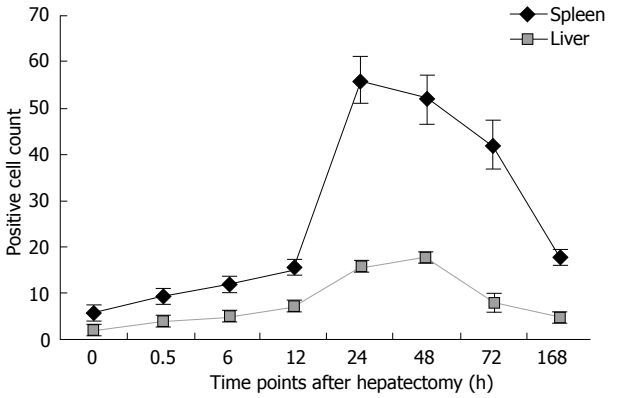

Figure 4 TACE-positive MNC in liver and spleen at various time points after hepatectomy. Data are mean ± SD, *n* = 7 animals in each group.

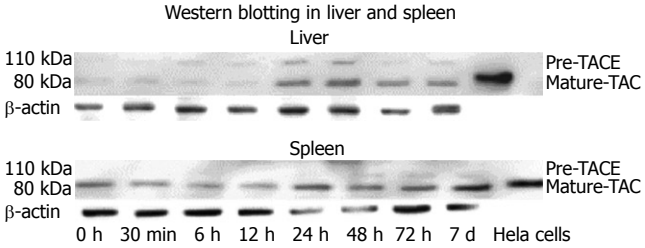

Figure 5 TACE protein level in rat liver and spleen following partial hepatectomy. Rat liver and spleen tissue samples taken at various time points were processed and subjected to Western blot analysis using anti-TACE antibodies. The hela cells were lysed and the proteins were loaded as a positive control. Expression of TACE in liver and spleen after hepatectomy is a time-dependent alteration, reaching a maximal level between 24 and 48 h.

using an anti-CD3 antibody. This experiment was done in light of the report that during liver regeneration, extrathymic $CD3^+$ T cells in liver are significantly activated in terms of increases in both proportion and absolute number. The feature demonstrated that the $CD3^+$ T lymphocytes partially overlapped TACE staining MNC (Figure 6).

DISCUSSION

Hepatocyte replication comprises two phases: priming and cell-cycle progression[12]. TNFα and interleukin-6 are the main cytokines triggering hepatocyte progression from G0 to G1. HB-EGF and TGFα play an important role in cell-cycle progression. Both TGFα and HB-EGF are ligands of the EGF family and are primary mitogens for hepatocyte proliferation in culture[13,14]. It is believed that the functions of TGFα and HB-EGF at least partially overlap during liver regeneration[15,16]. Enhanced expression of TGFα mRNA in hepatocytes peaks in 24 h and remains elevated for at least 48 h after hepatectomy[17]. TGFα anchored to the cell membrane in precursor form is cleaved by TACE and then binds to EGFR, which activates a phosphorylation cascade leading to DNA replication. The mitogenic cascade involves ERK1/2 and PKB. TNFα enlarges TACE activation by shedding the precursor of TGFα[18]. TGFα is produced by hepatocytes and functions through an autocrine mechanism. TGFα and EGF play a

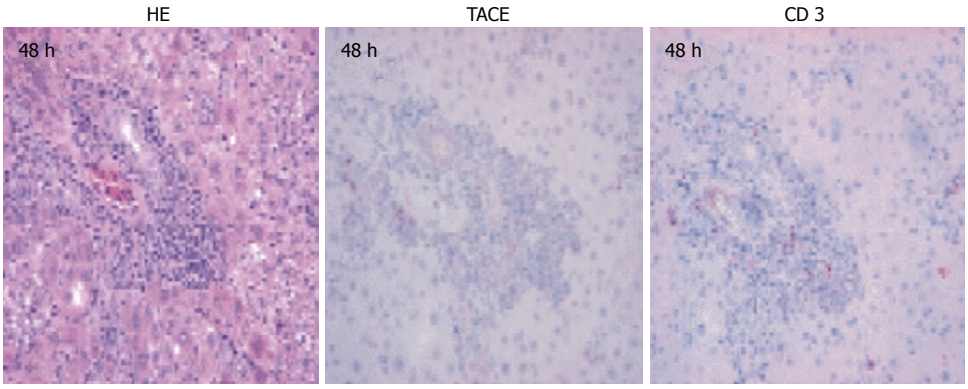

Figure 6 The serial paraffin sections of liver tissue at 48 h after hepatectomy were stained with HE and immunostained with anti-TACE antibodies and anti-CD3 antibodies. TACE-positive MNC partially overlapped with $CD3^+$ T lymphocytes (× 400).

major role in the progressive phases of liver regeneration after hepatectomy[19-21].

TACE is mediated by furin and related proprotein convertases. TACE was originally cloned and named for its ability to cleave and convert TNFα into a soluble form. Cells such as macrophages, lymphocytes, and monocytes, which all produce abundant TNFα, are believed to express TACE enzyme[22]. TACE is found to be involved in carcinogenesis by TGFα, and the HB-EGF-EGFR pathway. Distinct ADAM metalloproteinases regulate G protein-coupled receptor-induced cell proliferation and survival[23,24]. The TACE inhibitor TAPI-1 interferes with TGFα release into the culture medium and subsequent EGFR signalling through ERK1/2 and PKB, thereby blocking DNA replication[25]. These data substantiate the idea that TACE plays a significant role and forms a link between cytokine and growth factor pathways in cell proliferation.

In the present study, we examined the kinetic level of TACE expression and localization following partial hepatectomy *in vivo*. It demonstrated that the kinetics of TACE were relatively well synchronized with those of TGFα and hepatocyte proliferation[3,15,26]. After hepatectomy in rats, the first peak of DNA synthesis in hepatocytes occurs at about 24 h, with a smaller peak between 36 h and 48 h. The other cells of the liver enter into DNA synthesis at 48 h or later[12]. Although TACE is essential for cleaving TNFα and over-expression of TACE promotes inflammation by producing excessive soluble TNFα, our study shows that the kinetics of TACE expression are not compatible with those of TNFα in liver regeneration. TNFα increases abruptly and reaches a peak in the priming phase[12,27], but TACE rises to a peak from 24 h to 48 h post-hepatectomy. Such scenarios may suggest that TACE is involved in liver regeneration by pathways including TGFα-EGFR in the cell-cycle progressive phase, but not by the TNFα pathway. It was also reported that during liver regeneration, extrathymic $CD3^+$ T cells in the liver are significantly activated in terms of both increases in proportion and absolute number. This activation was observed at an early phase (d 2) of liver regeneration[28,29]. IL-1 and TNFα, which are produced by activated kupffer cells and sinusoidal endothelial cells, can induce the activation of T-cell differentiation. The mechanism of T-cell activation of hepatocyte proliferation is unequivocal[30]. In this research, immunohistochemical study using serial sections showed TACE-staining MNC partially overlaps with $CD3^+$ T lymphocytes. It is conceivable that TACE production and effect by paracrine may be a pathway of involvement in liver regeneration for activated $CD3^+$ T lymphocytes.

COMMENTS

Background

Tumor necrosis factor-alpha converting enzyme (TACE) is a kind of metalloprotease disintegrins that acts to solubilize a variety of substrates including tumor necrosis factor-α (TNFα), transforming growth factor-α (TGFα), epidermal growth factor (EGF) family and has been considered to involve in carcinogenesis by TGFα, heparin-binding epidermal growth factor-like growth factor (HB-EGF) pathway. It was also reported that the activity of several metalloproteinases increase during liver regeneration.

Research frontiers

Liver regeneration after partial hepatectomy is very intricate. It involves expression of multiple cytokines and growth factors such as HGF, TNFα TGFα and HB-EGF.

Innovations and breakthroughs

To date, there has been few studies on the correlation of TACE with liver regeneration after partial hepatectomy *in vivo*. To study the expression of TACE during liver regeneration, we investigated the liver and spleen tissues by a rodent model with partial hepatectomy. It demonstrated that TACE was produced by the activated $CD3^+$ T lymphocytes and the kinetic expression of TACE was well synchronized with that of TGFα and hepatocyte proliferation.

Applications

TACE is implicated in liver regeneration by the TGFa pathway that overlaps partially with carcinogenesis.

Peer review

The authors investigated the expression of TACE during liver regeneration in rats after 70% partial hepatectomy. They observed a time dependant expression with a peak between 24 h and 48 h. They located TACE to mononuclear cells. The authors conclude that TACE expression is synchronized with TNF-alpha and that TACE is expressed on mononuclear cells.

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S- Editor Zhu LH **L- Editor** Ma JY **E- Editor** Yin DH