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TEC protein tyrosine kinase is involved in the Erk signaling pathway induced by HGF

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ABSTRACT

Background/aims: TEC, a member of the TEC family of non-receptor type protein tyrosine kinases, has recently been suggested to play a role in hepatocyte proliferation and liver regeneration. This study aims to investigate the putative mechanisms of TEC kinase regulation of hepatocyte differentiation, i.e. to explore which signaling pathway TEC is involved in, and how TEC is activated in hepatocyte after hepatectomy and hepatocyte growth factor (HGF) stimulation.
Methods: We performed immunoprecipitation (IP) and immunoblotting (IB) to examine TEC tyrosine phosphorylation after partial hepatectomy in mice and HGF stimulation in WB F-344 hepatic cells. The TEC kinase activity was determined by *in vitro* kinase assay. Reporter gene assay, antisense oligonucleotide and TEC dominant negative mutant (TEC^{KM}) were used to examine the possible signaling pathways in which TEC is involved. The cell proliferation rate was evaluated by ^{3}H-TdR incorporation.
Results: TEC phosphorylation and kinase activity were increased in 1 h after hepatectomy or HGF treatment. TEC enhanced the activity of Elk and serum response element (SRE). Inhibition of MEK1 suppressed TEC phosphorylation. Blocking TEC activity dramatically decreased the activation of Erk. Reduced TEC kinase activity also suppressed the proliferation of WB F-344 cells. These results suggest TEC is involved in the Ras-MAPK pathway and acts between MEK1 and Erk.
Conclusions: TEC promotes hepatocyte proliferation and regeneration and is involved in HGF-induced Erk signaling pathway.

1. Introduction

TEC family of tyrosine kinases are non-receptor type protein tyrosine kinases (PTK) that consist of five members, namely TEC, Btk, Itk/Emt/Tsk, Bmx and Txk/Rlk [1]. These tyrosine kinases are known to have multiple cellular functions, especially in T-cell development differentiation [2]. TEC (tyrosine kinase expressed in hepatocellular carcinoma) is the first member of TEC family of tyrosine kinases that was cloned from liver cancer cells [3]. In mammals, TEC kinase is expressed predominantly in hematopoietic cells and hepatocytes.

While it's well demonstrated that TEC kinase is an integral component of T cell signaling and has distinct roles in T cell activation, the function of TEC in hepatocytes is not yet well understood. TEC has also been suggested to play a role in hepatocyte proliferation and liver regeneration [4]. We have previously shown that TEC is highly expressed in embryonic day 15–19 rat fetal livers but significantly decreased in adult and neonatal rat livers. Increased TEC expression was observed within 1 h after partial hepatectomy in rat [4]. These results suggested that TEC is an early, immediate response gene following partial hepatectomy (pHx) in rat. However, how TEC kinase functions during hepatocyte survival and growth, and how TEC kinase regulates signal transduction in hepatocyte remain to be determined.

Hepatocyte growth factor (HGF), a paracrine cellular growth, motility and morphogenic factor, has been extensively studied and is known to have a major role in embryonic organ development, wound healing and adult organ regeneration [5]. Specifically, previous studies have suggested that HGF is a potent mitogen for mature hepatocytes and acts as a hepatotropic factor for liver regeneration. Supporting this is the fact that HGF mRNA and HGF protein are rapidly and markedly increased in the liver and plasma of rats in various types of liver injuries [5]. A recent study using RNA interference to silence HGF and its receptor c-Met *in vivo* has further demonstrated the critical involvement of HGF/Met signaling in liver regeneration [6]. Therefore, we surmise that TEC kinase may regulate hepatocyte differentiation and is involved in HGF signaling.

Abbreviations: TEC, tyrosine kinase expressed in hepatocellular carcinoma; HGF, hepatocyte growth factor; pHx, partial hepatectomy; SRE, serum response element; SEAP, secreted alkaline phosphatase; MAPK, mitogen-activated protein kinase; ErK, extracellular signal-regulated kinases.

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In this study, to test above hypothesis, we examined the biological functions of TEC on hepatocyte proliferation in a mouse model of partial hepatectomy and in HGF-dependent WB F-344 hepatic cells. WB F-344 cells are from a diploid propagable rat hepatic epithelial cell line that shows potentials of progenitor cells of hepatocytes [7]. These cells are HGF-dependent, i.e. they hardly grow without HGF stimulation. We show here that TEC tyrosine kinase is phosphorylated after HGF stimulation in WB F-344 cells and after hepatectomy. Further mechanistic studies suggested that TEC kinase promote hepatocyte proliferation and is involved in the HGF-induced Erk-MAPK pathway.

2. Materials and methods

2.1. Cell line, animals, and reagents

Rat hepatic cell line WB F-344 was a generous gift from Dr. Yue Hang at the Chinese Academy of Sciences. Kunming mice were provided by the Experimental Animal Center of the Academy of Military Medical Sciences of China. Rat recombinant HGF was purchased from Sigma (Saint Louis, MO, USA). TEC antibody (Cat.# sc-1109), as well other antibodies were all purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Inhibitors of kinases MEK1, PI3K and P38 were all supplied by Promega (Madison, WI, USA). TEC^{KM}, a TEC kinase deficient mutant construct (the lysine-397 residue in the ATP-binding site of TEC kinase is replaced with methionine) [8] was a generous gift from Dr. Hiroyuki Mano at the Department of Molecular Biology, Jichi Medical School, Japan.

2.2. Partial hepatectomy

Eight-week-old mice were anesthetized with sodium pentobarbital (50 mg/kg). The median and left lateral lobes of the liver were excised and ligated at their stems [9]. The control mice were subjected to mock operation, i.e. laparotomy and brief manipulation of the intestines but not the liver with a cotton swab prior to wound closure [9]. The experimental and control mice were then sacrificed sequentially at 10, 20, 30, and 60 min after surgery and liver tissue lysates were prepared from these mice.

2.3. Immunoprecipitation and in vitro kinase assay

Cell or tissue lysates (2 mg) with anti-TEC antibody (1:100 dilution) at 4 °C for 1 h, and then incubated overnight with protein A-Sepharose 4B beads (Sigma, Saint Louis, MO, USA). The immune complexes were washed three times with lysis buffer and finally eluted with SDS–PAGE sample buffer.

For *in vitro* kinase assay the immune complexes were washed three times with lysis buffer and three times with kinase buffer before incubation with 0.37 MBq of γ-^{32}P-ATP at 30 °C for 15 min. The samples were then subjected to SDS–PAGE analysis.

2.4. Immunoblotting

Immunoblotting were performed as described in our previous articles. These antibodies include anti-TEC (1:1000 dilution), anti-Erk1/2 (1:2000 dilution), anti-phospho-Erk (1:2000 dilution), anti-AKT (1:1000 dilution), anti-phospho-AKT (1:1000 dilution), anti-p38 (1:1000 dilution), anti-phospho-p38 (1:1000 dilution), and the phosphorylation site specific anti-PY99 antibodies (1:1000 dilution).

2.5. Reporter gene assays for Elk and SRE

Reporter assays was used to determine Elk activation using the PathDetect *in vivo* reporting system (Stratagene, La Jolla, CA, USA). Basically, WB F-344 cells were co-transfected with pcDNA3.1(+)-TEC, pFR-luciferase, and one of the following expression plasmids: pFA-CREB, pFA-CHOP, pFA-Jun, or pFA-Elk-1 fusion plasmid. Transfected cells were cultured in serum-free DMEM for 6 h and then replaced by DMEM containing 1–2% FCS for another 32 h. The cells were then stimulated with HGF for 8 h. Cells were harvested and assayed for luciferase activity using the reagents provided (Promega, Madison, WI). SRE activity was examined using the BD Great EscA Pe^{TM} SEAP (secreted alkaline phosphatase) Vector System (BD Biosciences, San Diego, CA, USA). Transfection and SEAP assay were performed as the manufacturer suggested protocol.

2.6. Stimulation with TEC anti-sense/sense oligo nucleotides

TEC antisense oligo nucleotide (5′-AGACCGAGATGAATTTCA-3′) and sense control (5′-TGAA ATT CATCTCGGTCT-3′) were synthesized by Susbase company [10]. WB F-344 (6×10^5) were incubated in the presence or absence of 10 μM of synthetic oligonucleotide (anti-sense TEC or sense TEC) for 8 h. Then cells were stimulated with HGF (50 ng/ml) and incubated for additional 10–20 min. Whole cell lysates were prepared and subjected to IB to examine activities of Erk, AKT, and p38 kinases.

2.7. ^{3}H-TdR incorporation for scintillation countin

TEC anti-sense or sense oligo nucleotides were added and WB cells were cultured in serum-free medium for 10–12 h. Cells were then stimulated with HGF (50 ng/ml) for 24 h and 37 kBq of ^{3}H-TdR for additional 16 h. The radioactivity was measured in a liquid scintillation counter (Beckman, USA). The experiment was performed three times. Statistical analysis was conducted using Student's *t*-test and $p < 0.05$ shows statistical significance between HGF-treated and untreated group.

3. Results

3.1. Rapid tyrosine-phosphorylation of TEC by partial hepatectomy and HGF stimulation

To further investigate the role of TEC in hepatocyte proliferation and liver pathology, we established an *in vivo* mouse model of partial hepatectomy (pHx) and examined TEC tyrosine phosphorylation by immunoprecipitation with the liver tissue lysates prepared from the pHx or the control mice. As expected, tyrosine phosphorylation of TEC kinase was rapidly induced after partial hepatectomy, suggesting activation of TEC kinase (Fig. 1A). The phosphorylation level of TEC kinase was significantly higher than the basal level at 20–30 min after the surgery, while TEC phosphorylation in the control group remains unchanged. TEC expression remained unchanged in both group.

To determine whether TEC is involved in HGF induced signal transduction, WB F-344 cells lysates were subjected to anti-TEC IP followed by anti-phospho-tyrosine IB after HGF stimulation. The result showed that tyrosine phosphorylation of TEC kinase was significantly induced by HGF stimulation. The maximal level was shown at 10 min after stimulation (Fig. 1B).

3.2. TEC kinase activity is enhanced by HGF stimulation

Autophosphorylation is the phosphorylation of a kinase protein catalyzed by its own enzymatic activity. Analysis of kinase autophosphyorlation has been used to determine the status of kinase activation [11]. To measure TEC autophosphorylation with or without HGF stimulation, we performed *in vitro* kinase assay. The result showed that autophosphorylated of TEC was dramatically induced by HGF stimulation but not in the control group without HGF treatment (Fig. 1C).

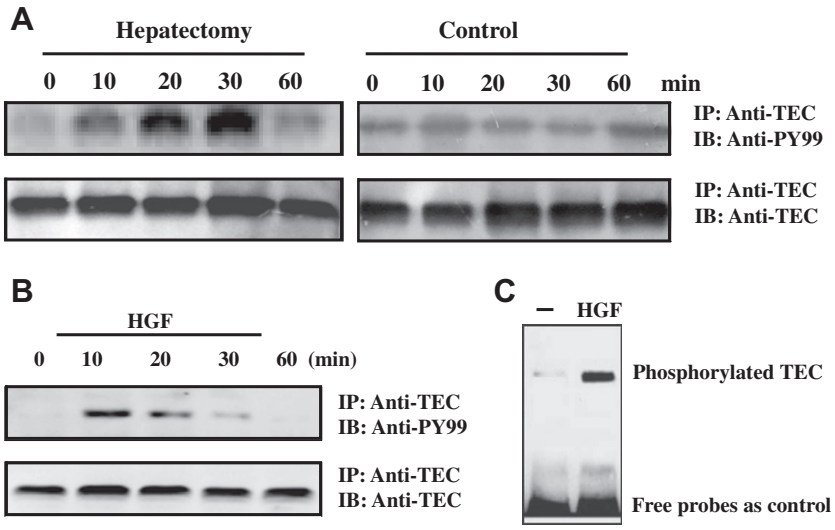

Fig. 1. TEC kinase phosphorylation and activity after partial hepatectomy in mice or stimulated by HGF. (A) Liver tissue lysates from mice undergone either sham-operation or partial hepatectomy were collected at 0, 10, 20, 30, and 60 min after surgery. The extracts were subjected to IP with TEC antibody followed by IB with anti-PY99. The results showed that tyrosine phosphorylation of TEC kinase was rapidly induced and was significantly higher than the basal level in mice at 20–30 min after partial hepatectomy. TEC phosphorylation in the control group remains unchanged. Blots were stripped and reblotted with anti-TEC antibody to examine TEC expression. (B) WB F-344 were starved for 24 h and stimulated by HGF for 0, 10, 20, 30, and 60 min before collection of the whole cell lysates. Cell lysates were subjected to anti-TEC IP followed by anti-phosphotyrosine IB. The result showed that tyrosine phosphorylation of TEC was significantly induced by HGF. The maximal level was shown at 10 min after stimulation. (C) TEC was immunoprecipitated from WB F-344 stimulated with or without HGF (50 ng/ml) for 10 min. The immune complexes were then used for *in vitro* kinase assay. The result showed TEC kinase activity was dramatically induced by HGF stimulation but not in the non-HGF group.

3.3. TEC kinase enhances the Erk pathway induced by HGF

HGF is involved in many signaling pathways, such as Erk-MAPK pathway, p38-MAPK pathway, PI3K pathway. To study how TEC is activated in hepatocytes after HGF stimulation, we performed *in vitro* reporter assay using the PathDetect *in vivo* reporting system. We examined the activation level of four reporter activator plasmids, pFA-CREB, pFA-Elk, pFA-CHOP and pFA-c-Jun by co-transfection with TEC and the pFR-luc plasmid in WB F-344 cells. Our results showed that TEC kinase specifically and significantly enhanced the pFA-Elk activity following HGF stimulation (lane *TEC + pFA-Elk*, Fig. 2A). The effect of TEC is also dose dependent with higher TEC concentration showing more Elk activity (Fig. 2B). We also assayed Elk activity in WB F344 cells that were transiently transfected with wild-type and kinase domain mutant TEC plasmid (TEC^{KM}). The results showed that over-expression of wild-type TEC further significantly increased HGF induced Elk activity, whereas overexpression of TEC^{KM} did not induce significant activation of Elk (Fig. 2C). These data suggest that TEC kinase can active Elk in HGF mediated MAPK signal pathway. Inactivation of TEC inhibits activation of Elk pathway induced by HGF in hepatocyte.

3.4. TEC promotes SRE (serum response element) activity after HGF stimulation

To study the putative mechanism of TEC involvement in the Erk/Elk signaling pathway, we examined its effect on SRE activation using the BD Great EscA Pe^{TM} SEAP reporter system. The results showed that the significantly upregulated SRE SEAP activity was observed in cells transfected with TEC expression plasmid and treated with HGF (Fig. 2D) suggesting that TEC kinase is involved in HGF induced SRE activation, although this effect may not be dose dependent (Fig. 2E). No detectable changes of SEAP activity was observed in TEC^{KM} transfected cells with or without HGF stimulation (Fig. 2F). We also examined TEC's role on AP-1 and NF-kB activation, no significant changes was observed (data not shown).

3.5. Mechanisms of TEC kinase in MAPK signaling pathway

3.5.1. TEC tyrosine phosphorylation is inhibited by MEK inhibitor

MEK1 is a known important intermediate between Raf and Erk. To identify where TEC kinase functions in the Erk signaling pathway, we studied TEC phosphorylation in WB cells using kinase inhibitors such as U0126 (MEK1 inhibitor), Ly294002 (PI3K inhibitor), and SB203580 (p38 inhibitor). The results showed that only U0126 significantly suppressed the HGF mediated TEC tyrosine phosphorylation (Fig. 2G). It suggests that TEC is specifically involved in the HGF mediated MEK1-Elk pathway and acts downstream of MEK1 kinase.

3.5.2. Erk1/2 activity is decreased by TEC anti-sense treatment

To further confirm the role of TEC kinase in the Erk/Elk pathway and to test whether TEC acts between MEK1 and Erk kinases, we examined the effect of TEC on Erk activation by suppressing its expression using antisense TEC. As shown in Fig. 3A, TEC expression is significantly decreased by antisense TEC, whereas no change of TEC expression was observed in the mock group or sense TEC group. We also examined the effect of TEC on activation of Erk, p38, and Akt kinases after HGF stimulation. The result showed that HGF induced activation of Erk1/2 was greatly reduced after treatment with TEC anti-sense oligo nucleotides. However, no significant changes of p38 and Akt activation were observed (Fig. 3B). These data strongly support a specific role of TEC kinase in HGF-mediated Erk activation. It also suggests that TEC is one of the regulators of Erk and functions between MEK and Erk in HGF induced MAPK pathway.

3.6. TEC plays a positive role in HGF induced WB F-344 cell proliferation

We examined the role of TEC on hepatocyte proliferation by ^{3}H-TdR incorporation assay on WB F-344 cells with sense- or anti-sense-TEC treatment. As seen in Fig. 3C, cells treated with TEC sense proliferate well after HGF stimulation. However, the cell

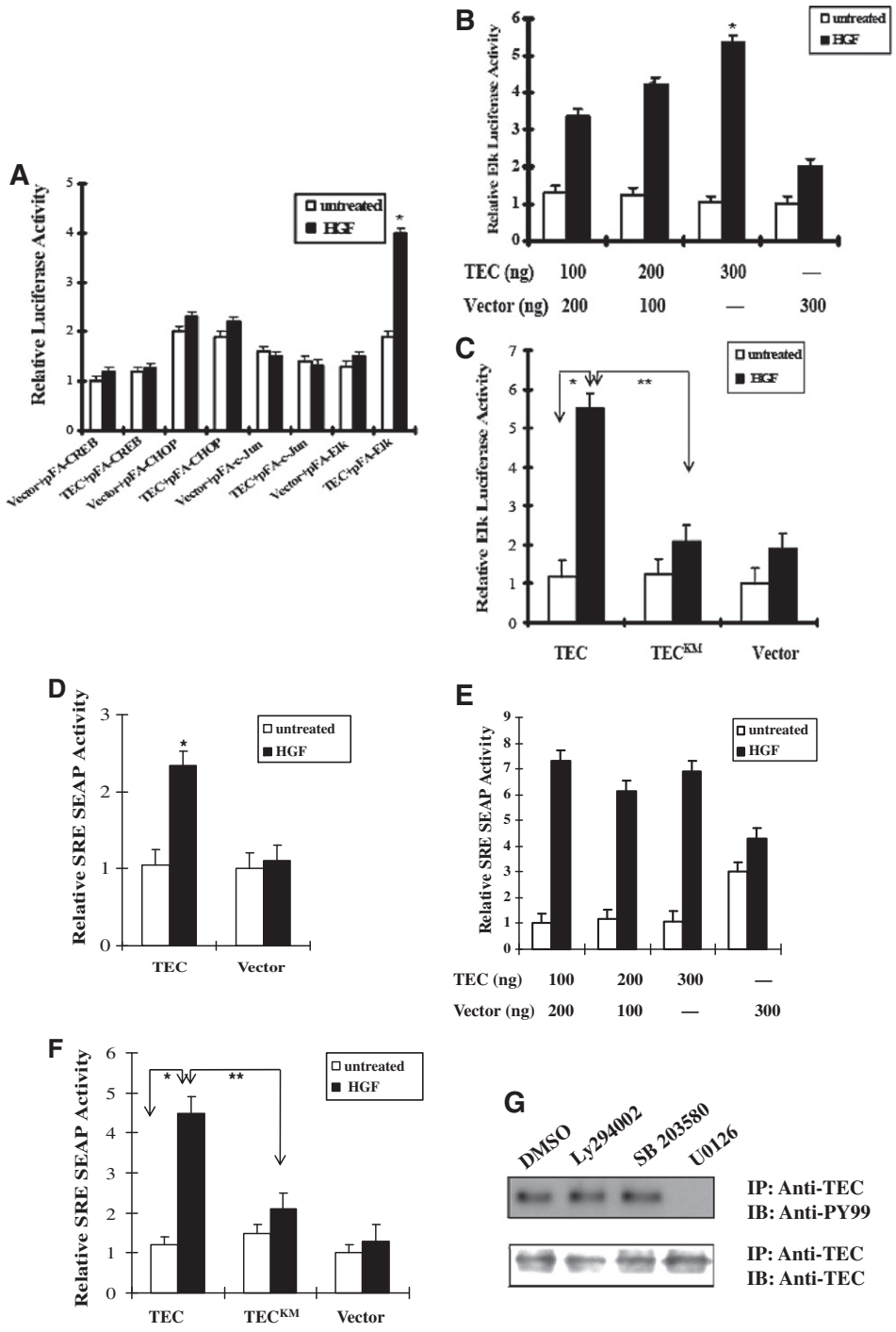

Fig. 2. TEC kinase is involved in HGF-induced activation of Elk and SRE. (A) WB F-344 were co-transfected with pcDNA3.1(+)-TEC, pFR-luciferase, and one of the following plasmids: pFA-CREB, pFA-CHOP, pFA-Jun, or pFA-Elk-1. Transfected cells were then stimulated with HGF (50 ng/ml) for 8 h. Then cells were harvested and assayed for luciferase activity. The result showed that TEC kinase specifically and significantly enhanced the pFA-Elk activity after HGF stimulation (lane *TEC + pFA-Elk*). (B) WB were transfected with pFR-luc reporter plasmid, pFA-Elk activity plasmid together with pcDNA3.1(+)-TEC or pcDNA3.1(+), respectively, at different amounts as indicated. The cells were starved cultured over 12 h and then stimulated with HGF (50 ng/ml) for 10 min. Elk activities were assayed as above. The result suggested the effect of TEC is dose dependent with higher TEC concentration showing more Elk activity. (C) WB F-344 were transfected with pFR-luc reporter plasmid, pFA-ELK activity plasmid and one of the following plasmid: pcDNA3.1(+)-TEC, pcDNA3.1(+)-TEC^{KM} or pcDNA3.1(+) vector. Elk activities were assayed as above. The result showed pcDNA3.1(+)-TEC increased Elk activation, whereas pcDNA3.1(+)-TEC^{KM} or pcDNA3.1(+) vector did not show obvious effect on the luciferase activity. (D) WB cells were co-transfected with SRE-SEAP reporter plasmid and either pcDNA3.1(+)-TEC or pcDNA3.1(+) vector as described in Materials and Method. SRE activities were assayed. The results showed that TEC significantly upregulated SRE activity after HGF stimulation. (E) WB cells were co-transfected with SRE-SEAP reporter plasmid as well as pcDNA3.1(+)-TEC or pcDNA3.1(+) at different amounts as indicated. SRE activities were assayed as above. Interestingly, the result suggested that TEC's effect on SRE activation is not dose dependent. (F) WB F-344 were transfected with SRE-SEAP reporter plasmid as well as pcDNA3.1(+)-TEC or pcDNA3.1(+)-TEC^{KM} or pcDNA3.1(+) empty vector. SRE activities were measured with or without HGF-stimulation. The result showed pcDNA3.1(+)-TEC increased SRE activation, whereas pcDNA3.1(+)-TEC^{KM} or pcDNA3.1(+) did not show obvious effect. (G) WB F-344 starved from serum for 24 h were treated with different kinase inhibitors (SB 203580, 15 μM; Ly294002, 20 μM; U0126, 20 μM; or DMSO) followed by HGF (50 ng/ml) treatment for 10 min. Whole cell lysates were prepared and subjected to IP using TEC antibody. The immunocomplexes were examined by IB with PY99 antibody. Blots were stripped and reblotted with anti-TEC antibody. The results showed TEC tyrosine phosphorylation was significantly suppressed by U0126, the MEK-1 inhibitor, suggesting that TEC is involved in HGF mediated MEK1-Elk signaling pathway and acts downstream of MEK1. Values were mean ± SD of three independent experiments and were compared with cells transfected with empty vector only. $^{*}P < 0.05$, $^{**}P < 0.01$.

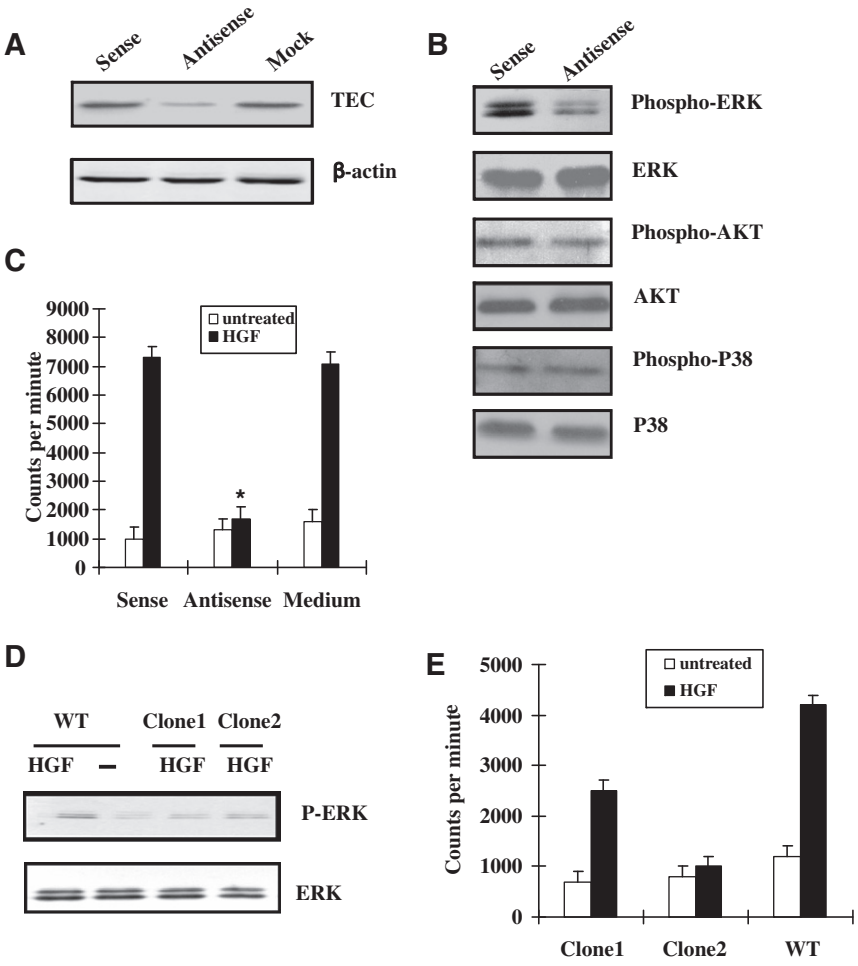

Fig. 3. Inhibition of TEC downregulated both HGF induced MAPK signaling pathway and hepatic cell proliferation. (A) WB F-344 cells (6×10^5) were incubated in the presence of 10 μM of synthetic oligonucleotide (anti-sense TEC or sense TEC) or medium alone (mock) for 8 h. Then cells were stimulated with HGF (50 ng/ml) and incubated for additional 10–20 min. Whole cell lysates were then examined by IB with anti-TEC antibody to test the expression of TEC. Blots were stripped and reblotted with anti-β actin to monitor protein loading. TEC expression is significantly decreased when cells were treated with antisense TEC, but not the ones with sense-TEC or with medium only. (B) WB F-344 cells were treated with 10 μM antisense TEC or sense TEC oligonucleotides. Whole cell lysates were prepared and examined by IB with following antibodies: anti-phospho-ERK1/2, anti-Erk, anti-phospho-AKT, anti-AKT, anti-phospho-p38, or anti-p38. The result showed that TEC anti-sense greatly reduced HGF induced Erk1/2 kinase activation compared to that treated with sense TEC. No significant changes of p38 and Akt activation were observed. (C) WB F-344 were treated with TEC antisense, TEC sense oligonucleotides, or medium alone for 12 h. The cells were starved cultured for 12 h and then stimulated with HGF (50 ng/ml) for another 24 h. WB F-344 proliferation mediated by HGF was measured by ^{3}H-TdR. The result showed when TEC expression was inhibited by TEC antisense, the cell proliferation rate was significantly decreased in the presence of HGF. (D) Wild type WB F-344 and two WB F-334 clones stably expressing TEC^{KM} (clone1 and 2) were stimulated with HGF for 10 min. Whole cell lysates were examined by IB with anti-phospho-ERK1/2 antibody. Blots were stripped and reblotted with anti-Erk antibody. The result showed that phosphorylation of Erk1/2 was significantly decreased in both clones suggesting dominant negative TEC decreased ERK1/2 activation. (E) WT WB and TEC^{KM} WB clones were starved cultured for 12 h followed by stimulation of HGF (50 ng/ml) for 24 h. Cell proliferative rate was examined by ^{3}H-TdR. As shown, the cell proliferation rate was significantly inhibited in both clone 1 and clone 2, suggesting dominant negative TEC decreased WB F-344 cell proliferation. Values were mean ± SD of three independent experiments and compared with sense oligo control. $^*P < 0.05$.

proliferation rate decreased significantly when TEC antisense was added into WB F-344 cells in the presence of HGF. To further study if the effect of TEC on cell proliferation is kinase catalytic activity dependent, we treated WB F-344 cells with TEC^{KM}. Two WB cell clones stably expressing TEC^{KM} were selected and treated with HGF. Activation of Erk1/2 was then examined and the result showed that phosphorylation of Erk1/2 was significantly decreased in both clones (Fig. 3D). As expected, the cell proliferation rate was also significantly inhibited in two clones (Fig. 3E). These results demonstrated that TEC is involved in hepatocyte proliferation mediated by HGF.

4. Discussion

In this paper, we reported further characterization of the effect of TEC (a non-receptor tyrosine kinase) and its correlation with HGF on hepatocyte proliferation and liver regeneration. We have previously shown that TEC mRNA level is significantly increased within 4 h of rat PHx [12]. Another study using a rat model has suggested that HGF-related signal transduction cascades contribute to hepatocyte proliferation and are initiated within 1 min after PHx [13]. Interestingly, there are also reports which suggested that no TEC expression level changes were observed after 72 h of partial hepatectomy [1,14]. These results suggest that TEC's responses to liver injures is not consistent and the data are generated from studies using rat models. We have shown here that tyrosine phosphorylation of TEC kinase was rapidly induced after partial hepatectomy of mice and the significant high level phosphorylation of TEC kinase was detected 20–30 min after the surgery. The tyrosine phosphorylation of TEC kinase was also significantly induced by HGF stimulation in WB F-344 hapatic cells. Our data confirmed that TEC kinase is an early-immediate response gene and

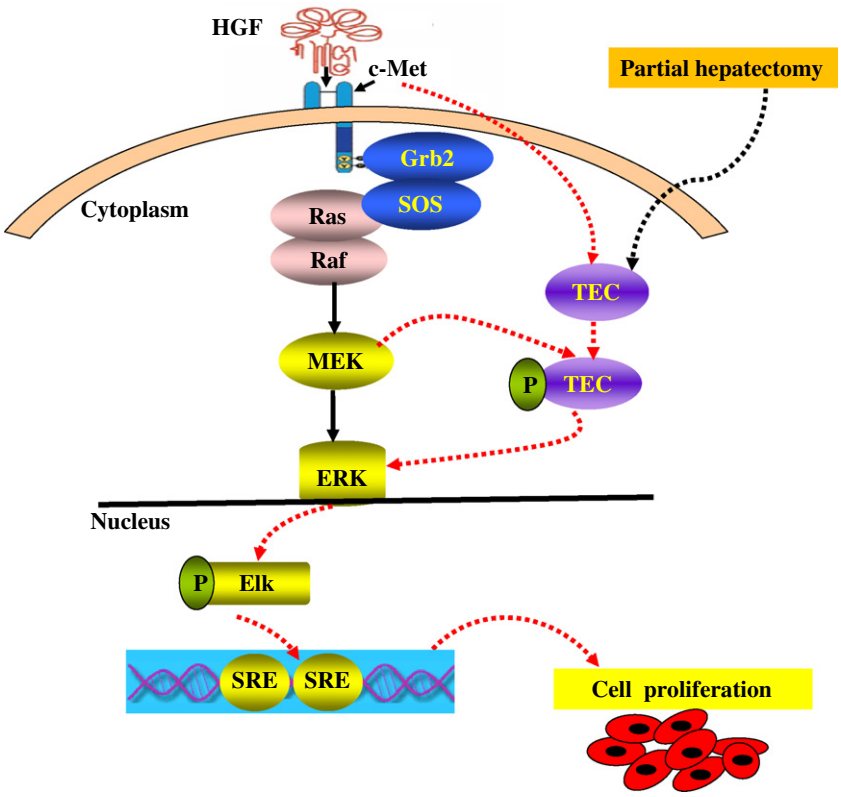

Fig. 4. TEC in HGF induced Erk signaling pathway. Illustrated is the canonical Ras/MAPK pathway that has been described previously. Briefly, HGF binds to c-Met receptor and activates the tyrosine kinase activity by triggering the following GRB2 and SOS signaling cascades. Then it causes the activation of Ras and then the MEK and Erk kinase, leading to activation of a number of effectors, notably transcription factors, which play a direct role in cell proliferation or differentiation. Our study shows that TEC is rapidly tyrosine-phosphorylated and activated by HGF-stimulation or partial hepatectomy. TEC promotes the Erk-MAPK signaling pathway and acts downstream of MEK1 kinase but upstream of ERK kinase in HGF signaling.

demonstrated for the first time that TEC is involved in HGF-induced signaling and hepatocyte proliferation in mouse acute liver injury (PHx). This suggests that TEC indeed plays an important role in early phase of liver regeneration.

We have also shown that TEC kinase dose-dependently enhanced the Elk activity following HGF stimulation and TEC kinase is involved in HGF induced SRE (serum response element) activation. On the other hand, decreased expression of TEC kinase, suppressed TEC activity, or TEC^{KM} significantly reduced activation of Erk by HGF, whereas inhibition of MEK resulted in decreased TEC tyrosine phosphorylation. These observations suggest that TEC kinase is specifically involved in the HGF mediated Raf-MEK1-Elk-Erk pathway and acts downstream of MEK1 kinase. The Erk signaling pathway involves activation of many transcriptional factors including Elk1, a c-Fos proto-oncogene regulator. Previously, it has been demonstrated that Elk can activate SRE and control *C-fos* expression, an oncogene that plays important roles in cell growth, differentiation and cellular transformation processes [15]. Our further studies demonstrated that TEC participates in WB F-344 proliferation and this effect may be due to activation of SRE activity. This is consistent with previous report that overexpression of TEC strongly induces transcriptional activation of the *C-fos* proto-oncogene in blood cell [16]. Our results may indicate that TEC kinase contribute to malignant proliferation in liver cancer cells.

In summary, we have demonstrated that TEC is rapidly tyrosine-phosphorylated and activated by HGF-stimulation or partial hepatectomy. TEC promotes the Erk (but not p38 or Akt) signaling pathway and acts downstream of MEK1 kinase. We have also shown that TEC enhance activation of SRE which suggest that TEC is involved in liver cell proliferation. Previously, the HGF/c-Met pathway has been demonstrated to be the earliest signaling pathways activated after partial hepatectomy. It has also been shown that *in vivo* silencing of c-Met and HGF mRNA resulted in decreased proliferation kinetics associated with liver regeneration. Based on these observations, we propose following working diagram outlining the putative mechanisms and the signaling pathway, the HGF/*C-met* regulated Ras/MAPK or Erk signaling pathway, that TEC might be involved during its regulation of hepatocyte differentiation/proliferation (Fig. 4). Further characterization of TEC and its related proteins during cell proliferation and differentiation may help to identify novel therapeutic targets for liver regeneration associated with liver injury and liver cancer.

Conflict of interest statement

All authors have no conflicts of interest.

Acknowledgments

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References

[1] H. Mano, Tec family of protein-tyrosine kinases: an overview of their structure and function, Cytokine Growth Factor Rev. 10 (3–4) (1999) 267–280.

[2] M. Felices, M. Falk, Y. Kosaka, L.J. Berg, Tec kinases in T cell and mast cell signaling, Adv. Immunol. 93 (2007) 145–184.
[3] H. Mano, F. Ishikawa, J. Nishida, H. Hirai, F. Takaku, A novel protein-tyrosine kinase, Tec is preferentially expressed in liver, Oncogene 5 (1990) 1781–1786.
[4] W. Xu, S. Wang, G. Wang, H. Wei, Xiao Yang, Identification and characterization of differentially expressed genes in the early response phase during the liver regeneration, Biochem. Biophys. Res. Commun. 278 (2) (2000) 318–325.
[5] T. Nakamura, Hepatocyte growth factor as mitogen, motogen and morphogen, and its roles in organ regeneration, Princess Takamatsu Symp. 24 (1994) 195–213.
[6] S. Paranjpe, W.C. Bowen, A.W. Bell, K. Nejak-Bowen, J.H. Luo, G.K. Michalopoulos, Cell cycle effects resulting from inhibition of hepatocyte growth factor and its receptor c-Met in regenerating rat livers by RNA interference, Hepatology 45 (6) (2007) 1471–1477.
[7] W.K. Kaufmann, C.I. Behe, V.M. Golubovskaya, L.L. Byrd, C.D. Albright, K.M. Borchet, S.C. Presnell, W.B. Coleman, J.W. Grisham, G.J. Smith, Aberrant cell cycle checkpoint function in transformed hepatocytes and WB F-344 hepatic epithelial stem-like cells, Carcinogenesis 22 (8) (2001) 1257–1269.
[8] H. Mano, Y. Yamashita, A. Miyazato, Y. Miura, K. Ozawa, Tec protein-tyrosine kinase is an effector molecule of Lyn protein-tyrosine kinase, FASEB J. 10 (5) (1996) 637–642.
[9] J. Kountouras, P. Boura, N.J. Lygidakis, Liver regeneration after hepatectomy, Hepatogastroenterology 48 (38) (2001) 556–562.
[10] A. Kitanaka, H. Mano, M.E. Conley, D. Campana, Expression and activation of the nonreceptor tyrosine kinase Tec in human B cells, Blood 91 (3) (1998) 940–948.
[11] R.E. Joseph, D.B. Fulton, A.H. Andreotti, Mechanism and functional significance of Itk autophosphorylation, J. Mol. Biol. 373 (5) (2007) 1281–1292.
[12] Si-Ying Wang, Fei-Fei Li, Hong Zheng, Ke-Ke Yu, Fang Ni, Xiao-Ming Yang, Cheng-Kui Qu. Rapid induction and activation of Tec tyrosine kinase in liver regeneration, J. Gastroenterol. Hepatol. 21 (2006) 668–673.
[13] D.B. Stolz, W.M. Mars, B.E. Petersen, T.H. Kim, G.K. Michalopoulos, Growth factor signal transduction immediately after two-thirds partial hepatectomy in the rat, Cancer Res. 59 (16) (1999) 3954–3960.
[14] H. Mano, K. Mano, B. Tang, M. Koehler, T. Yi, D.J. Gilbert, Expression of a novel form of Tec kinase in hematopoietic cells and mapping of the gene to chromosome 5 near Kit, Oncogene 8 (2) (1993) 417–424.
[15] H.M. Zhang, L. Li, N. Papadopoulou, G. Hodgson, E. Evans, M. Galbraith, M. Dear, S. Vougier, J. Saxton, P.E. Shaw, Mitogen-induced recruitment of ERK and MSK to SRE promoter complexes by ternary complex factor Elk-1, Nucl. Acids Res. 36 (8) (2008) 2594–2607.
[16] Y. Yamashita, S. Watanabe, A. Miyazato, Tec and Jak2 kinase cooperate to mediate cytokine-driven activation of c-fos transcription, Blood 91 (1998) 1496–1507.