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TGFβ enforces activation of eukaryotic elongation factor-2 (eEF2) via inactivation of eEF2 kinase by p90 ribosomal S6 kinase (p90Rsk) to induce mesangial cell hypertrophy

Falguni Das [a], Nandini Ghosh-Choudhury [b,d], Balakuntalam S. Kasinath [a,b], Goutam Ghosh Choudhury [a,b,c,*]

[a] Department of Medicine, University of Texas Health Science Center at San Antonio, TX, USA
[b] VA Research, South Texas Veterans Health Care System, San Antonio, TX, USA
[c] Geriatric Research, Education and Clinical Research, South Texas Veterans Health Care System, San Antonio, TS, USA
[d] Department of Pathology, University of Texas Health Science Center at San Antonio, TX, USA

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ABSTRACT

eEF2 phosphorylation is under tight control to maintain mRNA translation elongation. We report that TGFβ activates eEF2 by decreasing eEF2 phosphorylation and simultaneously increasing eEF2 kinase phosphorylation. Remarkably, inhibition of Erk1/2 blocked the TGFβ-induced dephosphorylation and phosphorylation of eEF2 and eEF2 kinase. TGFβ increased phosphorylation of p90Rsk in an Erk1/2-dependent manner. Inactive p90Rsk reversed TGFβ-inhibited phosphorylation of eEF2 and suppressed eEF2 kinase activity. Finally, inactive p90Rsk significantly attenuated TGFβ-induced protein synthesis and hypertrophy of mesangial cells. These results present the first evidence that TGFβ utilizes the two layered kinase module Erk/p90Rsk to activate eEF2 for increased protein synthesis during cellular hypertrophy.

1. Introduction

Transforming growth factor-β (TGFβ) is an enforcer of diverse functions including immune regulation, wound healing, cell proliferation and migration, apoptosis and extracellular matrix expansion [1]. The active TGFβ dimer elicits these biological activities by binding to type II receptor, which phosphorylates the type I receptor at GS domain, a 30 amino acid segment, located immediately upstream of the kinase domain [1]. Activated type I receptor in the tetrameric complex recruits the TGFβ receptor-specific R-Smads2/3 to phosphorylate at the C-terminal serine residues. Phosphorylated Smads dissociate from the cytoplasmic retention protein SARA to expose the nuclear localization signal and translocate to the nucleus and associate with common Smad, Smad4 [2]. The heterodimeric Smad complex then associates with other DNA binding transcription factors and transcriptional coactivator or corepressor to induce or repress gene expression [1,2]. However, Smad-independent TGFβ signaling also coexists with the canonical Smad pathway. These pathways include Erk1/2, JNK and p38 MAPK [1]. We and others have recently shown that TGFβ activates phosphatidylinositol 3 kinase/Akt cascade to increase expression of collagen I and fibronectin, two profibrotic genes [3,4].

TGFβ contributes to nephropathy, which consists of whole kidney and glomerular hypertrophy and altered glomerular hemodynamics [5]. Increased glomerular volume correlates with hypertrophy of mesangial cells, which constitute a third of the glomerular cell population that leads to accumulation of matrix proteins and glomerulosclerosis [6]. We have recently reported involvement of TGFβ-induced PI 3 kinase/Akt signaling in the development of glomerular, especially mesangial cell hypertrophy, during the progression of diabetic nephropathy [7].

Cellular hypertrophy is defined by the increase in protein synthesis with minimal changes in DNA synthesis [8]. About 95% of mRNAs are translated in a cap-dependent manner, where translation initiation phase acts as the rate-limiting step [9]. We have recently demonstrated a role of TGFβ-stimulated TORC1 activity in inactivation of the translation repressor 4EBP-1, which contributes to formation of initiation complex 4F by releasing the eIF4E to induce cap-dependent mRNA translation [9,10]. However, it is during the elongation phase that peptide synthesis occurs in both cap-dependent and -independent translation. The 93 kD eukaryotic elongation factor 2 (eEF2), when bound to GTP in its N-terminal segment, mediates the translocation of aminoacyl tRNA during elongation [11,12]. Phosphorylation of threinone-56 in this domain

* Corresponding author. Address: Department of Medicine, University of Texas Health Science Center at San Antonio, Mail Code 7882, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900, USA.
E-mail address: choudhuryg@uthscsa.edu (G.G. Choudhury).

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inhibits its ribosome binding activity thereby inactivating it [11]. eEF2 kinase (eEF2K) that phosphorylates eEF2 at Thr-56 is unusual in that it is not a member of the Ser–Thr or Tyr kinase superfamily [13]. The N-terminus of eEF2K contains a calmodulin binding site and its activity is normally dependent upon Ca^{2+} and calmodulin [11]. Several serine residues including Ser-366 have been identified in the eEF2K, which are phosphorylated by different stimuli that specifically activate kinases such as S6 kinase, Rsk1/2, SAPK4, CDC2, PKA and MK-2 [11,14,15]. Insulin- and IGF-1-mediated activation of receptor tyrosine kinases induces phosphorylation of eEF2K at Ser-366, which impairs its activity towards eEF2, resulting in increased elongation of protein synthesis [11,16]. How eEF2 phosphorylation is regulated by eEF2K in response to serine–threonine kinase receptor activation is not known.

In the present study, we demonstrate that TGFβ induces dephosphorylation of eEF2 with concomitant increase in phosphorylation of eEF2K. These phosphorylation events are sensitive to activation of Erk1/2 and its target p90 ribosomal S6 kinase (Rsk). Finally, we demonstrate that TGFβ-stimulated p90Rsk contributes to mesangial cell protein synthesis and hypertrophy.

2. Materials and methods

2.1. Materials

Recombinant TGFβ was purchased from R & D Systems. Phospho-eEF2 (Thr-56), eEF2, phospho-eEF2K (Ser-366), eEF2K, phospho-Erk1/2 (Thr-202/Tyr-204), Erk1/2, phospho-p90Rsk (Ser-380) and p90Rsk antibodies were obtained from Cell Signaling Technology. Anti-actin antibody was obtained from Sigma. Anti-HA antibody was purchased from Covance. Dominant negative Erk2 expression vector has been described previously [17]. Expression of dominant negative Erk2 blocks the kinase activity of both Erk1 and Erk2 [18]. Dominant negative p90Rsk1 plasmid (pKH3 Rsk1 K112/464R) was kindly provided by Dr. John Blenis, Harvard Medical School. Dominant negative Rsk1 may inhibit the kinase activity of other Rsk isoforms. However, Rsk1 is predominantly expressed in kidney [19].

2.2. Cell culture and transfection

Rat kidney glomerular mesangial cells were grown as described previously [4,7,10]. Mesangial cells were transfected with vector or indicated expression plasmids using Fugene as described [10,20,21]. Using this reagent, we routinely observe 60–80% transfection efficiency in mesangial cells [10].

2.3. Immunoblotting

Mesangial cells were lysed in RIPA buffer (20 mM Tris–HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA, 1 mM $Na3VO_4$, 1 mM PMSF, 0.1% protease inhibitor cocktail and 1% NP-40) and the cell debri were separated by centrifugation to yield supernatant, which was used to determine protein concentration as described [4,7,10,20]. Equal amounts of proteins were separated by SDS–polyacrylamide gel electrophoresis followed by transfer of the separated proteins to PVDF membrane for immunoblotting with indicated antibodies as described [4,7,10,20].

2.4. Protein synthesis assay and measurement of mesangial cell hypertrophy

Protein synthesis was determined using ^{35}S-methionine incorporation as described [7,10,21]. The cellular hypertrophy is defined by increase in protein synthesis without minimal changes in DNA synthesis, i.e., in the absence of cell proliferation. Therefore, hypertrophy of mesangial cells was measured as the ratio of total protein to number of cells as described [7,10,21].

3. Results

3.1. TGFβ activates eEF2 in Erk1/2-sensitive manner

The phosphorylation of eEF2 at Thr-56 inactivates its function to induce elongation phase of mRNA translation [11,12]. To study eEF2 phosphorylation in response to TGFβ, we immunoblotted lysates of TGFβ-incubated mesangial cells with phospho-eEF2 antibody. TGFβ decreased eEF2 phosphorylation in a time-dependent manner (Fig. 1A and Supplemental Fig. S1A). The eEF2 phosphorylation at Thr-56 is mediated by the highly specific eEF2K; activity of eEF2K is impaired by phosphorylation at Ser-366 [11]. Incubation of mesangial cells with TGFβ significantly increased phosphorylation of eEF2K (Fig. 1B and Supplemental Fig. S1B). Note that, dephosphorylation of eEF2 followed the same kinetics as that of eEF2K phosphorylation and hence inactivation (Fig. 1A and B).

TGFβ is known to activate the non-Smad signaling, including Erk1/2 [1]. TGFβ increased phosphorylation of Erk1/2 in mesangial cells (Supplementary Fig. S2). We examined the involvement of

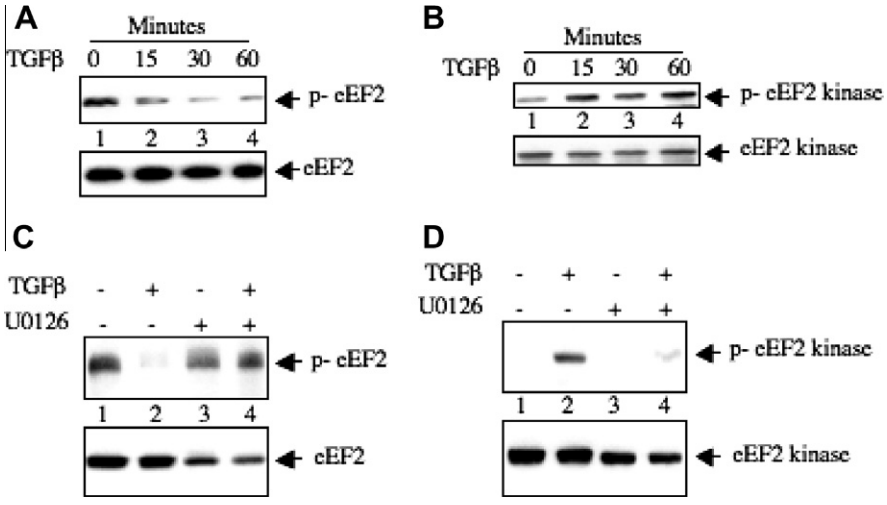

Fig. 1. TGFβ decreases phosphorylation of eEF2 by increasing phosphorylation of eEF2K in Erk1/2-dependent manner. (A and B) Lysates of mesangial cells incubated with 2 ng/ml TGFβ for the indicated periods of time were immunoblotted with the indicated antibodies. (C and D) Mesangial cells were treated with 5 μM U0126 for 1 h prior to incubation with TGFβ for 15 min. The cell lysates were immunoblotted with the indicated antibodies. The quantification of these data is provided in Supplementary Fig. S1.