Cooperative induction of transmembrane prostate androgen induced protein TMEPAI/PMEPA1 by transforming growth factor-β and epidermal growth factor signaling

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journal or publication title	Biochemical and biophysical research communications
volume	456
number	2
page range	580-585
year	2015-01
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URL	http://hdl.handle.net/2241/00124159

doi: 10.1016/j.bbrc.2014.11.107

Fig. S1 (Azami S. *et.al.*)

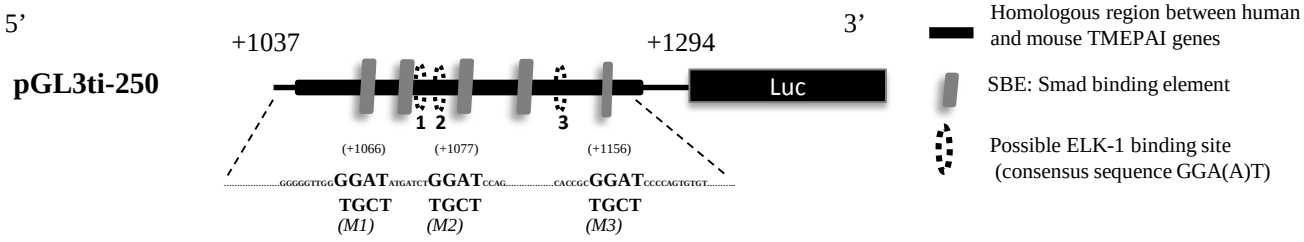

Fig. S1: Schematic representation of pGL3ti-250 reporter containing the ELK-1 binding sites. Three ELK-1 –binding sites possesses consensus sequence GG(A)T at position +1066, +1077, and +1156 within the first intron of the TMEPAI gene. The binding site mutants were made by changing GGAT to TGCT.

Fig. S2 (Azami S. *et.al.*)

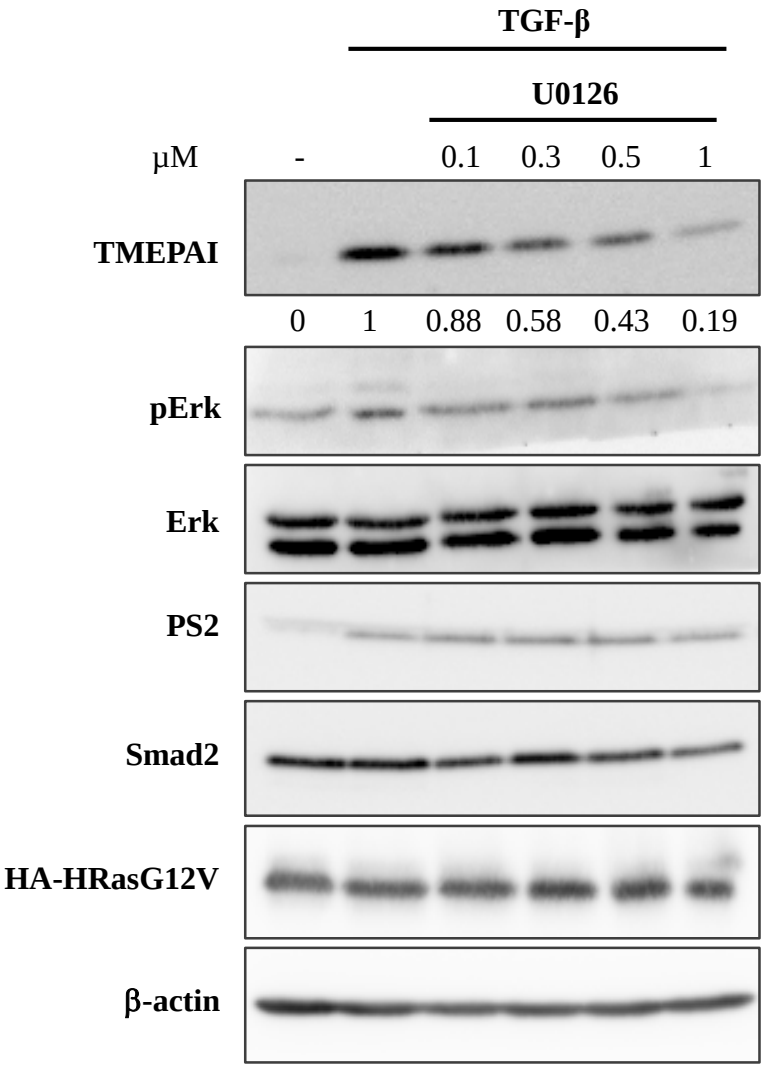

Fig. S2: EGF/Ras/MAPK pathway enhances TGF-β induced-TMEPAI expression. HaCaT-RasG12V cells were treated with MEK inhibitor U0126, as indicated, 1 h before TGF-β (0.5 ng/ml) stimulation for 8 h. Cell lysates were subjected to immunoblot analysis and detected with anti-TMEPAI antibody (9F10). The levels of phosphorylated Erk, total ERK, phosphorylated Smad2, Smad2 and HA-RasG12V were also detected to examine the effects of TGF-β and U0126 treatments. β-actin was used as the loading control. Relative expression levels of TMEPAI/β-actin were detected by densitometry and indicated below the panels.

Fig. S3 (Azami S. *et.al.*)

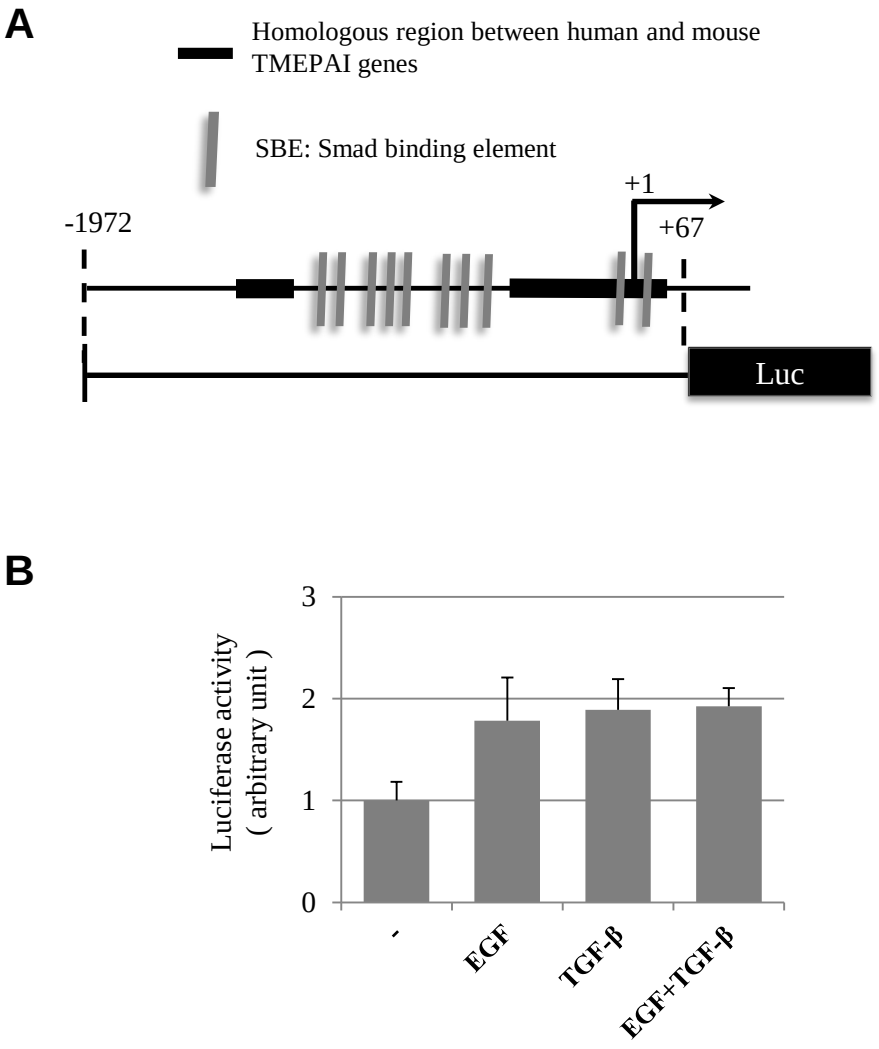

Fig. S3: Transcriptional activity of TMEPAI promoter upon EGF and TGF-β stimulation. (A) Schematic representation of the TMEPAI 5'-promoter luciferase reporter construct -1972TMEPAI-luc. The nucleotide numbers of mouse TMEPAI gene was shown with the transcriptional initiation site as +1. (B) HepG2 cells were transfected with -1972TMEPAI-luc and stimulated with EGF (10 ng/ml), TGF-β (0.1 ng/ml), or both EGF (10 ng/ml) and TGF-β (0.1 ng/ml) for 18 h.

Fig. S4 (Azami S. *et.al.*)

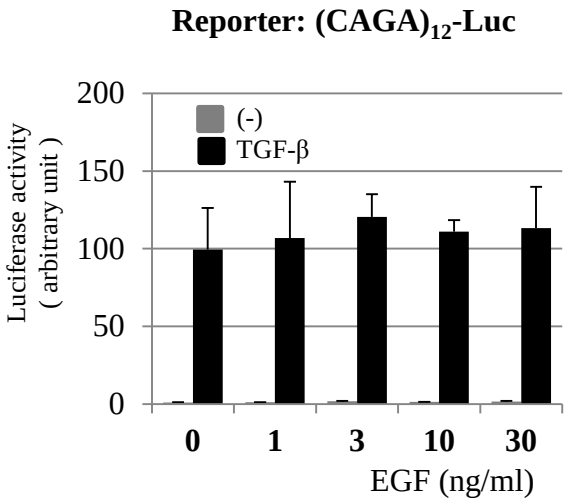

Fig. S4: The effect of EGF on Smad dependent transcription. HepG2 were transfected with $(CAGA)_{12}$-Luc and treated with EGF for 18 h with indicated concentrations in the presence or absence of TGF-β (0.1 ng/ml) stimulation.

Fig. S5 (Azami S. *et.al.*)

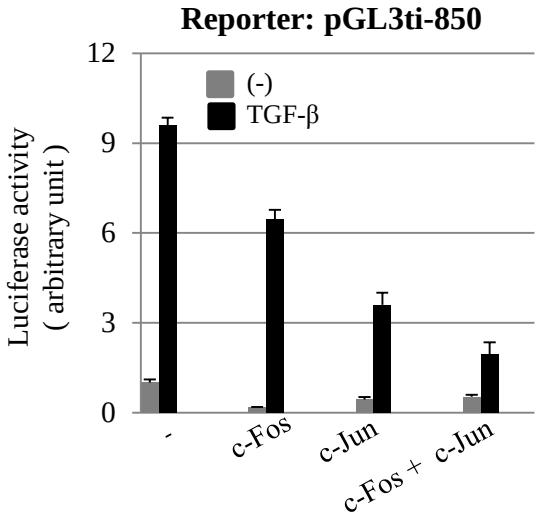

Fig. S5: The involvement of AP-1 transcriptional factor on TMEPAI expression. HepG2 cells were transfected with pGL3ti-850 together with c-Fos and/or c-Jun as indicated in the presence or absence of TGF-β (0.1ng/ml) for 18h.

Fig. S6 (Azami S. *et.al.*)

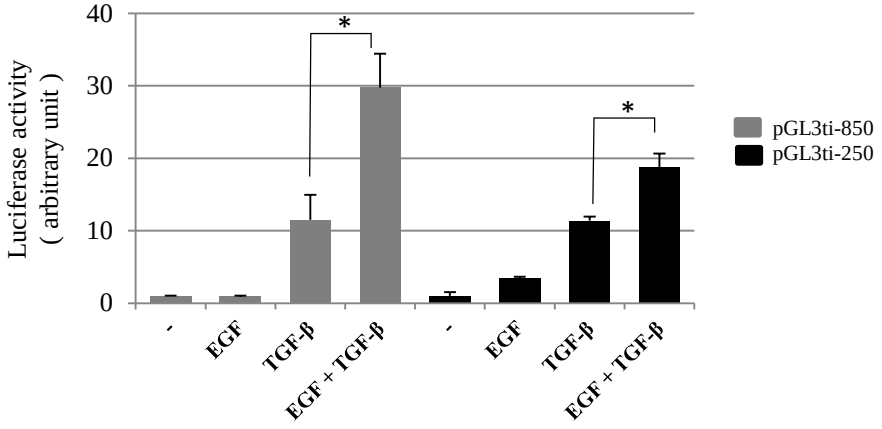

Fig. S6: Transcriptional activity of the first intron of TMEPAI gene upon EGF and TGF-β stimulation. HepG2 cells were transfected with pGL3ti-850-luc or pGL3ti-250-luc, and stimulated with EGF (10 ng/ml), TGF-β (0.1 ng/ml), or both EGF (10 ng/ml) and TGF-β (0.1 ng/ml) for 18 h. (*$P < 0.05$)