Upregulation of dual-specificity phosphatase-26 is required for transforming growth factor β1(TGFβ1)-induced Epithelial-mesenchymal transition in A549 and PANC1 cells

Sabire Guler1 · Berrin Zik1 · Abdullah Yalcin2

Received: 30 May 2022 / Accepted: 19 August 2022 / Published online: 2 September 2022
© The Author(s), under exclusive licence to Springer Nature B.V. 2022

Abstract

Background  Transforming Growth Factor β (TGFβ) proteins are potent inducers of the epithelial-mesenchymal transition (EMT) in tumor cells. Although mitogen-activated protein kinase (MAPK) family has been shown to be involved in TGFβ-induced EMT, role of Dual Specificity Phosphatases (DUSP), key regulators of MAPK activity, in TGFβ-induced EMT is largely unknown.

Methods and results  Real-time qPCR analyses were performed to determine the effect of TGFβ1 on expression of EMT genes and DUSP proteins in the non-small cell lung cancer model A549 and pancreatic adenocarcinoma model PANC1 cells. Western blot analyses were conducted to study the changes in protein levels of EMT proteins and select DUSP proteins, as well as phosphorylations of MAPK proteins upon TGFβ1 stimulation. Small interfering RNA (siRNA) was utilized to reduce expressions of DUSP genes. We observed that the EMT phenotype coincided with increases in phosphorylations of the MAPK proteins ERK1/2, p38MAPK, and JNK upon TGFβ1 stimulation. Real-time qPCR analysis showed increases in DUSP15 and DUSP26 mRNA levels and Western blot analysis confirmed the increase in DUSP26 protein levels in both A549 and PANC1 cells treated with TGFβ1 relative to control. Silencing of DUSP26 expression by siRNA markedly suppressed the effect of TGFβ1 on E-cadherin and mesenchymal genes in the cells.

Conclusions  Data provided suggest that TGFβ1 modulates the expression of DUSP genes and that upregulation of DUSP26 may be required for TGFβ1-promoted EMT in A549 and PANC1 cells. Further studies should be carried out to elucidate the requirement of individual DUSPs in TGFβ1-associated EMT in tumor cells.

Keywords  TGFβ1 · Epithelial-mesenchymal transition · Dual-specificity phosphatases · MAPK · DUSP26

Introduction

Epithelial-mesenchymal transition (EMT) is characterized by the transdifferentiation of apico-basal polarized epithelial cells into mesenchymal spindle-shaped cells [1]. The EMT process, which is known to have important effects on embryogenesis and organogenesis, has been shown to play an effective role in cancer invasion and metastasis, as well as chemotherapeutic resistance [2, 3]. EMT is a highly dynamic and multistep process that can be induced by a variety of proteins and cytokines, such as Transforming Growth Factor β [4].

TGFβ proteins (TGFβ1-3) are potent inducers of the EMT in tumor cells that are associated with tumor invasiveness and chemoresistance [5–7]. TGFβ activates the EMT via SMAD-dependent and SMAD-independent pathways [8]. In the SMAD-dependent pathway, TGF-activated SMAD complex migrates to the nucleus, where it regulates the expression of EMT transcription factors, including the SNAI1 and SNAI2 genes, which encode Snail and Slug proteins, respectively. The EMT transcription factors bind to the promoter of the prototypical epithelial gene CDH1 (encoding E-Cadherin), suppressing its transcription [9]. SMAD proteins can also directly promote the expression of some mesenchymal genes such as fibronectin and vimentin [10]. Furthermore,
in a SMAD-independent manner, the TGFβ signaling pathway modulates the activity of proteins that belong to the Mitogen-Activated Protein Kinase (MAPK) family, including ERK1/2, JNK, and p38 MAPK, which regulates and potentiates the EMT induced by SMAD proteins [11].

Dual specificity phosphatases (DUSPs) are a subfamily of protein phosphatases that dephosphorylates MAPKs. DUSPs comprise the typical DUSPs, which contain a domain that interacts with MAPKs (KIM-kinase interaction motif) such as DUSP1 and 4, and the atypical DUSPs, which have no discernible MAPK-interacting domain, such DUSP14 and 26 [12]. Given the known role of MAPKs in the EMT in tumor cells, various DUSP members have been implicated in invasive and chemoresistant phenotype of some tumor types, with some DUSP members promoting invasiveness and chemoresistance while others suppressing these properties, suggesting distinct functions. For example, DUSP26 displays both tumour-suppressive and -promoting properties depending on the context [13]. In pancreatic cancer and glioma tissues, DUSP26 mRNA has been shown to be overexpressed relative to normal tissues (https://www.proteinatlas.org/ENSG00000133878-DUSP26/pathology). Given the large numbers of DUSPs that have overlapping and non-overlapping functions in tumor cells, elucidation of the role of individual DUSPs in a context- and stimuli dependent manner may help us better understand the molecular underpinnings of oncogenic processes such as EMT [14].

In this study, we aimed to profile the expressions of typical and atypical DUSPs in response to TGFβ1, and to determine the requirement of DUSPs in TGFβ1-induced EMT in the non-small cell lung cancer model A549 and pancreatic adenocarcinoma model PANC1 cells.

**Material and methods**

**Cell culture and treatment**

The non-small cell lung cancer cell line A549 (CRM-CCL-185) and the pancreatic duct epithelial carcinoma cell line PANC1 (CRL-1469) cells were purchased from ATCC and cultured in 10% fetal bovine serum (Gibco, 10270106) supplemented RPMI (Biological Industries, 01-100-1A) and DMEM (Gibco, 2241121) respectively. Cells were grown at 37 °C in 5% CO2. The recombinant TGFβ1 (PeproTech, 100-21C) was diluted in PBS containing 0.2% bovine serum albumin and added to culture at 5 ng/ml concentration.

**Real-time quantitative PCR**

RNA isolation (ThermoFischer, K0732) and cDNA synthesis (ThermoFischer, 4368814) were carried out following the manufacturer’s instructions. Real time quantitative PCR (qPCR) analyses were performed on StepOne Plus (ThermoFischer, U.S.A) using gene expression master mix (Promega, A6101) and gene-specific TaqMan probes (ThermoFisher). The probes used were Snail (Hs00195591_m1), E-cadherin (4331182), Slug (Hs01023895_m1), N-cadherin (Hs01023894_m1), Fibronectin (Hs01549976_m1), DUSP1 (Hs00610256_g1), DUSP2 (Hs01091226_g1), DUSP4 (Hs01027785_m1), DUSP5 (Hs00244839_m1), DUSP6 (Hs04329643_s1), DUSP7 (Hs00997002_m1), DUSP8 (Hs00792712_g1), DUSP9 (Hs01046584_g1), DUSP10 (Hs00200527_m1), DUSP16 (Hs00411837_m1), DUSP3 (Hs0115776_m1), DUSP11 (Hs01061375_m1), DUSP12 (Hs00170898_m1), DUSP13 (Hs00969203_m1), DUSP14 (Hs01877076_s1), DUSP15 (Hs01566654_m1), DUSP18 (Hs01036622_g1), DUSP19 (Hs00369901_m1), DUSP21 (Hs00254403_s1), DUSP22 (Hs00414885_m1), DUSP23 (Hs00367783_m1), DUSP26 (Hs00225167_m1), DUSP27 (Hs01367756_m1), DUSP28 (Hs01374134_m1), and GAPDH (Hs03929097_g1). GAPDH was used as the internal control for normalization of CDNA. The relative fold change in mRNA expressions was determined using the 2-ΔΔCt method [15].

**Western blot analysis**

Western blot analysis was performed as previously described [16]. Briefly, cells were washed twice in cold PBS and lysed with lysis buffer for setting up protein lysates. The proteins were resolved by 4–12% SDS-PAGE gel electrophoresis and transblotted onto polyvinylidene fluoride membrane. After blocking with 5% nonfat dry milk in Tris-buffered saline, membranes were incubated with primary antibodies Snail (Cell Signaling, 3879), Slug (Cell Signaling, 9585), E-cadherin (Cell Signaling, 3195), ERK1/2 (Cell Signaling, 4695), phospho-ERK1/2 (Cell Signaling, 4370), JNK (Cell Signaling, 9252), phospho-JNK (Cell Signaling, 4668), p38 (Cell Signaling, 8690), phospho-p38 (Cell Signaling, 4511), DUSP26 (My BioSource, MBS8531048), α-Tubulin (Cell Signalling, 2144), β-actin (Cell Signaling, 3700) overnight at 4 °C. HRP-conjugated goat anti-rabbit (Cell Signaling, 7074) or anti-mouse (Cell Signaling, 7076) secondary antibodies were used. Immunoreactive bands were visualized by using chemiluminescence (Luminata Forte HRP Substrate, Millipore) in ChemiDoc™ MP Imaging System (Bio-Rad). The intensities of the protein bands were quantified using ImageJ software.

**Fig. 1** TGFβ1 induces EMT and activates MAPK pathway in PANC1 cells. A Morphology of cells in light microscope. B Real-time qPCR analyses, C Immunofluorescence analyses, D Western blot analyses of EMT markers. DAPI was used for nuclear staining. E Western blot analyses of phosphorylated and total levels of MAPK proteins (JNK, ERK1/2, and p38 MAPK) in control and TGFβ1-treated cells. Data were presented as the mean ± s.d. of three replicates. Ctrl, Control, *p < 0.05, compared to Ctrl
A) Bright field images show a comparison between control (Ctrl) and TGFβ1 treated conditions.

B) mRNA expression (fold of control) analysis with Ctrl and TGFβ1 treated groups. Significant differences are indicated with asterisks.

C) Immunofluorescence images for E-cadherin, Slug, and Fibronectin, comparing Ctrl and TGFβ1 treatments.

D) Western blot analysis for E-cadherin, Snail, Slug, and β-actin. Protein bands are labeled with molecular weights.

E) Western blot analysis for p-ERK1/2, ERK1/2, pJNK, JNK, Pp38, and P38. Protein bands are labeled with molecular weights.
ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997–2018.).

**Immunofluorescence**

The seed cells on coverslips were fixed in 4% formaldehyde and the method was applied as described in manufacturer’s instructions (Cell Signaling). The slides were incubated with the primary antibodies E-cadherin (Cell Signaling, 3195), Slug (Cell Signaling, 9585), Fibronectin (Sigma, F7387) at 4 °C overnight and probed with Alexa-Fluore 488-conjugated goat anti-rabbit antibody (Cell Signaling, 4412) for fluorescence detection. The images were visualized under EVOS Imaging System (ThermoFischer, USA.). ImageJ was used to analyze staining intensities of the images.

**SiRNA transfections**

To silence DUSPs mRNAs, previously designed and commercially available (ThermoFisher) siRNA molecules were used (DUSP1, 4427038; DUSP15, 4427037; and DUSP26,
s35360). As a negative control, cells were transfected with the universal siRNA molecule, which has no homology in the human genome (ThermoFisher, 4390846). Transfection of siRNAs was performed with Lipofectamine RNAiMAX (ThermoFisher, 4390846) when cells reached approximately 50% confluency at the time of transfection. The final siRNA concentration was 10 nM.

**Statistical analyses**

Experiments were performed three times. Data were expressed as the mean ± s.d. of triplicate measurements of a single experiment, except for mRNA levels which were done in duplicate. Independent samples t-test was used to assess a statistical significance. $p < 0.05$ was considered significant. Statistical analyses were performed using IBM SPSS Statistics 23.

**Results**

**TGFβ1 induces EMT and activates MAPK pathway**

We recently had shown that TGFβ1 induced an EMT phenotype in the non-small cell lung cancer model A549 cells [17]. To determine if TGFβ1 also induces EMT in the pancreatic adenocarcinoma model PANC1 cells in our hands, we incubated these cells with TGFβ1 (5 ng/ml) for 48 h, and then the cells were morphologically examined under a microscope. We observed the transdifferentiation of the predominantly epithelial cells into fibroblast-like mesenchymal cells (Fig. 1A). We then performed real-time qPCR analyses on EMT markers and transcription factors that are known to be regulated by TGFβ1 [5]. As seen in Fig. 1B, TGFβ1 significantly induced Snail, N-cadherin and fibronectin mRNA levels, while it suppressed E-cadherin mRNA levels. We then performed immunofluorescence analyses in PANC1 cells incubated with TGFβ1 and observed a decrease in the E-cadherin protein on the cell membrane and increases in Slug and fibronectin proteins (Fig. 1C). We then conducted Western blot analyses and demonstrated a decrease in the E-cadherin protein and an increase in the EMT transcription factor Snail protein, indicating EMT induction by TGFβ1 in PANC1 cells (Fig. 1D). We next studied MAPK pathway activity by analyzing the phosphorylated and total levels of the MAPK proteins ERK1/2, p38MAPK, and JNK. TGFβ1 treatment enhanced the phosphorylated forms of all the MAPK proteins studied (Fig. 1E). We also confirmed the EMT in A549 cells by TGFβ1 (Supplementary Fig. 1).

**TGFβ1 causes changes in expressions of DUSPs**

We conducted real-time qPCR analyses to profile the expression levels of all the 14 known typical DUSP and 9 of the atypical DUSP genes that are predicted to regulate MAPK activity [18]. Changes in expressions of DUSP mRNAs were shown as supplementary information (Supplementary Fig. 2). We had previously reported increases in DUSP1, DUSP7, DUSP15 and DUSP26 mRNAs in A549 cells (Fig. 2A) [17]. Given that the DUSP26 mRNA displayed the highest change in response to TGFβ1, we investigated DUSP26 protein levels by Western blot and demonstrated the elevated protein expression by TGFβ1 (Fig. 2B). Here, we found that, as in A549 cells, TGFβ1 induced DUSP15 and DUSP26 mRNA levels in PANC1 cells (Fig. 2C); however, unlike A549 cells, TGFβ1 reduced DUSP1 mRNA in PANC1 cells relative to controls cells (Fig. 2C). We then performed Western blot analysis and confirmed the induction of the DUSP26 protein in PANC1 cells (Fig. 2D).

**Suppression of DUSP26 partially reverses EMT induced by TGFβ1**

In order to determine the requirement of the increases in the above DUSPs in TGFβ1-induced EMT, we transfected A549 cells with siRNAs targeting DUSP1, DUSP15 and DUSP26 individually, or as various combinations, and 48 h later, E-cadherin mRNA levels were analyzed by real-time qPCR. We found only DUSP26 siRNA transfection alone was able to reverse, at least in part, the suppressive effect of TGFβ1 on E-cadherin expression (Fig. 3A). We verified the decrease in DUSP26 mRNA by siRNA (Fig. 3B). To further validate the requirement of DUSP26 in TGFβ1-induced EMT, we analyzed Snail, Slug, fibronectin, and N-cadherin mRNAs by real-time qPCR, and observed that DUSP26 silencing partially suppressed the increases in Snail, Slug, and N-cadherin mRNAs by TGFβ1 (Fig. 3C). We then conducted Western blot analysis to confirm the effect of DUSP26 silencing on increased Snail expression in TGFβ1-treated cells (Fig. 3D). Taken together, these data suggest that the induction of DUSP26 is required for the promotion of the EMT by TGFβ1.

The same analyses were conducted in PANC1 cells. PANC1 cells were transfected with DUSP15, DUSP26 siRNAs individually, or as combination, and 48 h later, real-time qPCR analysis was carried out to analyzed E-cadherin mRNA levels. We observed that, as in A549 cells, DUSP26 siRNA alone was able to reduce the suppression of E-cadherin mRNA by TGFβ1 (Fig. 4A). We confirmed the
efficacy of DUSP26 siRNA in suppressing DUSP26 mRNA (Fig. 4B). DUSP26 silencing markedly reduced TGFβ1-induced expression of Snail and fibronectin mRNA levels in PANC1 cells (Fig. 4C). We then showed that DUSP26 silencing reduced the level of the Snail protein, as determined by Western blot (Fig. 4D).

Discussion

MAPKs are increasingly being recognized as key players in mediating the effects of TGFβ1 signaling in the EMT in tumor cells [19]. However, the potential role of the protein phosphatases that counteract MAPK activity, namely DUSPs, is less appreciated in the EMT. In this study, we aimed to determine the effect of TGFβ1 on the expression levels of DUSPs in non-small cell lung cancer and pancreatic adenocarcinoma EMT models.

As expected and previously shown, the EMT was successfully induced by TGFβ1 in both A549 [17], and PANC1 cells, as assessed by changes in cell morphology (Fig. 1A). Changes in the morphology were coincided with changes in the expression levels of the EMT proteins, including increases in the expression of mesenchymal genes such as Snail and decreases in the expression of the epithelial gene E-cadherin.

The effect of TGFβ1 on the EMT is mediated by SMAD-dependent and -independent pathways [20]. In the SMAD-independent pathway, one or several of the MAPKs ERK1/2, JNK, and p38 MAPK have been implicated in the EMT process depending on the cellular context and other factors such as the stage of oncogenic transformation [21]. In our recent study [17], we had demonstrated that TGFβ1 signaling only increased the phosphorylation of ERK1/2 in A549 cells. In this study, we observed that the phosphorylation levels of all the MAPK members studied—ERK1/2, JNK, and p38 MAPK—were elevated by TGFβ1 in PANC1 cells, supporting the notion that the effect of TGFβ1 signaling on MAPK activity is context-dependent [22, 23]. For example, while ERK was shown to be phosphorylated in A549 cells, JNK was phosphorylated in PANC1 and CFPAC1 cells upon TGFβ1 [24, 25].

Although the effects of DUSPs expressed in different cell types on MAPK activities have been examined in previous studies, only a handful of DUSPs has been linked to the EMT in tumor cells [26–28]. Studies that suggest a role for DUSPs indicate differential effects for DUSP members in the EMT and related phenotypes. For example, while DUSP1 is required for the stem cell phenotype, DUSP4 and DUSP6 inhibits it in breast cancer cells undergoing EMT [28]. In pancreatic ductal adenocarcinomas, DUSP2 inhibition is required for the EMT that is mediated by ERK1/2 activation [29, 30]. The tumor suppressive effect of DUSP6 was additionally observed in esophageal squamous cell carcinomas and nasopharyngeal carcinomas, as well as in various EMT-related oncogenic assays [31].

We found significant decreases in DUSP1 and DUSP6 in PANC1 cells upon TGFβ1 supplementation. However, TGFβ1 induced DUSP1 expression in A549 cells, suggesting a differential effect on DUSP1 expression in these cells. TGFβ1 markedly induced DUSP15 and DUSP26 expressions in both cell lines. Overexpression of some DUSP members have been linked to the malignant phenotype in some cancer types [32]. In this study, we conducted silencing experiments in order to determine the specific effect of DUSP1, DUSP15 and DUSP26 TGFβ1-promoted EMT. Individual and combined transfections of siRNAs targeting DUSP1, DUSP15 and DUSP26 indicated that the incuded expression of DUSP26 may be necessary for TGFβ1-promoted EMT in both A549 and PANC1 cells. However, our observation that DUSP26 silencing alone significantly reversed TGFβ1’s effect on E-cadherin expression and this effect was lost with the combination of DUSP1 and DUSP15 silencing in A549 cells (Fig. 3A) and DUSP15 silencing in PANC-1 cells (Fig. 4A) suggests that DUSP1 and DUSP15 may counteract the function of DUSP26 in TGFβ1-promoted EMT. DUSP26 has recently been shown to be overexpressed in non-small cell cancer cells and its inhibition suppresses proliferation, migration and invasion and EMT in the cells [33]. Our study, to our knowledge, is first to show an association between TGFβ1-induced EMT and DUSP26. DUSP26 has a tumor suppressing or promoting effect, depending on the context and stage of tumor progression [34, 35]. Patterson et al. [34] demonstrated that DUSP26 mRNA levels were lower in neuroblastoma cell lines compared to normal adrenal tissue, suggesting a tumor-suppressor function for DUSP26. In accordance with this notion, low DUSP26 mRNA
expression has been shown to be highly associated with decreased patient survival in neuroblastoma primary samples [36]. On the other hand, Shi et al. [35] demonstrated that DUSP26 knockdown decreases cell proliferation in both neuroblastoma cell lines and a xenograft model, suggesting a tumor-promoting function. In anaplastic thyroid cancer cells that express low levels of DUSP26, ectopic DUSP26 expression promotes cell growth through inhibition of p38-mediated apoptosis, while DUSP26 targeting in cells that had amplified DUSP26 reduces proliferation, suggesting a pro-oncogenic role [37]. Our current study supports the notion that DUSP26 is involved in TGFβ1-induced EMT, as assessed by an increase in E-cadherin and a decrease in Snail levels in A549 cells and an increase in E-cadherin and decreases in Snail and fibronectin levels in PANC1 cells treated with TGFβ1 relative to control cells.

Given the involvement of the EMT in malignant phenotypes, our data indicating the reduced EMT upon DUSP26 silencing in TGFβ1-stimulated cells also support an oncogenic role for DUSP26, as demonstrated by previous studies [35]. However, whether MAPKs, p38 MAPK in particular—a known substrate for DUSP26 [18]—are involved in this process remains to be determined, as MAPK-independent functions of DUSPs have also been reported [34].

In conclusion, we demonstrate that TGFβ1 modulates the expression of DUSP1, DUSP15 and DUSP26 genes, and that upregulation of DUSP26 may be required for TGFβ1-promoted EMT in A549 and PANC1 cells. This data warrants further studies aimed at elucidating the requirement of individual DUSPs in TGFβ1-associated EMT in tumor cells.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11033-022-07893-1.

Author contributions Study was designed by [SG] and [AY]. Material preparation, data collection and analysis were performed by [SG] and [BZ]. The manuscript was written by [SG] and edited by [AY]. All authors read and approved the final manuscript.

Funding This work was supported by Scientific and Technological Research Council of Turkey [Sabire Guler] (Grant # 118Z092). Türkiye Bilimsel ve Teknolojik Araştırma Kurumu, 118Z092, Sabire GULER

Declarations

Conflict of interest Sabire Guler has received research from The Scientific and Technological Research Council of Turkey (Grant Number: 118Z092). Berrin Zülc declares that she has no conflict of interest. Abdullah Yalcin declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Derynck R, Weinberg RA (2019) EMT and cancer: more than meets the eye. Dev Cell 49(3):313–316. https://doi.org/10.1016/j.devcel.2019.04.026
2. Staalduijn VJ, Baker D, Dijke PT, van Dam H (2018) Epithelial-mesenchymal-transition-inducing transcription factors: new targets for tackling chemoresistance in cancer? Oncogene 37:6195–6211. https://doi.org/10.1038/s41388-018-0378-x
3. Yang J, Weinberg RA (2008) Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. Dev Cell 14(6):818–829. https://doi.org/10.1016/j.devcel.2008.05.009
4. Gavert N, Ben-Ze’ev A (2008) Epithelial-mesenchymal transition and the invasive potential of tumors. Trends Mol Med 14:199–209. https://doi.org/10.1016/j.tolmed.2008.03.004
5. Moustakas A, Heldin CH (2012) Induction of epithelial-mesenchymal transition by transforming growth factor β. Semin Cancer Biol 22(5–6):446–454. https://doi.org/10.1016/j.semcancer.2012.04.002
6. Baba AB, Rah B, Bhat GR, Mushtaq I, Parveen S, Hassan R, Hameed Zargar M, Afroze D (2022) Transforming growth factor-beta (tgf-β) signaling in cancer-a betrayal within. Front Pharmacol 13:791272. https://doi.org/10.3389/fphar.2022.791272
7. Gu S, Feng XH (2018) TGF-β signaling in cancer. Acta Biochim Biophys Sin 50(10):941–949. https://doi.org/10.1093/abbs/gmy092
8. Lamouille S, Xu J, Derynck R (2014) Molecular mechanisms of epithelial–mesenchymal transition. Nat Rev Mol Cell Biol 15(3):178–196. https://doi.org/10.1038/nrm3758
9. Puisieux A, Brabletz T, Caramel J (2014) Oncogenic roles of EMT-inducing transcription factors. Nat Cell Biol 16(6):488–494. https://doi.org/10.1038/ncb2976
10. Nawshad A, Medici D, Liu CC, Hay ED (2007) TGFβ3 inhibits E-cadherin gene expression in palate medial-edge epithelial cells through a Smad2-Smad4-LEF1 transcription complex. J Cell Sci 120(9):1646–1653. https://doi.org/10.1242/jcs.003129
11. Boye A, Kan H, Wu C, Jiang Y, Yang X, He S, Yang Y (2015) MAPK inhibitors differently modulate TGF-β/Smad signaling in HepG2 cells. Tumour Biol 36(5):3643–3651. https://doi.org/10.1007/s13277-014-3002-x
12. Huang CY, Tan TH (2012) DUSPs, to MAP kinases and beyond. Cell Biosci 2:24. https://doi.org/10.1186/2045-3701-2-24
13. Thompson EM, Stoker AW (2021) A review of DUSP26: structure, regulation and relevance in human disease. Int J Mol Sci 22(2):776. https://doi.org/10.3390/ijms22020776
14. Nunes-Xavier C, Roma-Mateo C, Rios P, Tarrega C, Cejudo-Marin R, Taberner L, Pulido R (2011) Dual-specificity MAP kinase phosphatases as targets of cancer treatment. Anticancer Agents Med Chem 11:109–132. https://doi.org/10.2174/1871511794941190

15. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(−ΔΔC(T)) Method. Methods 25(4):402–408. https://doi.org/10.1006/meth.2001.1262

16. Daniel JMP (2010) Methodological considerations for improving Western blot analysis. J Pharmacol Toxicol Methods 61(2):171–217. https://doi.org/10.1016/j.vascn.2009.12.001

17. Guler S, Altunok TH, Sarioğlu A, Zik B, Asmaz D, Kayapunar N, Sonmez O, Erbaykent Tepedelen B, Yalcin A (2022) Overexpression of dual-specificity phosphatases 4 and 14 attenuates transforming growth factor β1-induced migration and drug resistance in A549 cells in vitro. Biochem Bioph Res Commun 606:35–41. https://doi.org/10.1016/j.bbrc.2022.03.090

18. Patterson KI, Brummer T, O’Brien PM, Daly RJ (2009) Dual-specificity phosphatases: critical regulators with diverse cellular targets. Biochem J 418:475–489. https://doi.org/10.1042/bj20082234

19. Davies M, Robinson M, Smith E, Huntley S, Prime S, Paterson I (2005) Induction of an epithelial to mesenchymal transition in human immortal and malignant keratinocytes by TGF-β1 involves MAPK, Smad and AP-1 signalling pathways. J Cell Biochem 95(5):918–931. https://doi.org/10.1002/jcb.20458

20. Derynck R, Zhang Y (2003) Smad-dependent and Smad-independent pathways in TGF-β family signalling. Nature 425:577–586. https://doi.org/10.1038/nature02006

21. Yue J, Mulder KM (2000) Activation of the mitogen-activated protein kinase pathway by transforming growth factor-β. Methods Mol Biol 142:125–131. https://doi.org/10.1385/1-59259-053-5:125

22. Zhang J, Jiang N, Ping J, Xu L (2021) TGF-β1-induced autophagy activates hepatic stellate cells via the ERK and JNK signalling pathways. Int J Mol Med 47:256–266. https://doi.org/10.3892/ijmm.2020.4778

23. Yu L, Hebert MC, Zhang YE (2002) TGF-β receptor-activated p38 MAP kinase mediates Smad-independent TGF-β responses. EMBO J 21:3749–3759. https://doi.org/10.1093/emboj/cdf366

24. Yinyun N, Sisi W, Xiangxiu W, Guonian Z, Xuemei C, Yu D, Wei M (2018) Cucurbitacin I induces pro-death autophagy in A549 cells via the ERK-mTOR-STAT3 signaling pathway. J Cell Biochem 119(7):6104–6112. https://doi.org/10.1002/jcb.26808

25. Luo J, Xiang Y, Xu X, Fang D, Li D, Ni F, Zhu X, Chen B, Zhou M (2018) High glucose-induced ROS production stimulates proliferation of pancreatic cancer via inactivating the JNK pathway. Oxid Med Cell Longev. https://doi.org/10.1155/2018/6917206

26. Lang R, Hammer M, Mages J (2006) DUSP meet immunology: dual specificity MAPK phosphatases in control of the inflammatory response. J Immunol 177(11):7497–7504. https://doi.org/10.4049/jimmunol.177.11.7497

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.