TGF-β-induced transgelin promotes bladder cancer metastasis by regulating epithelial-mesenchymal transition and invadopodia formation

Zhicong Chen\textsuperscript{a,b,c,\*}, Shiming He\textsuperscript{a,b,c,\*}, Yonghao Zhan\textsuperscript{a,b,c,d,e,\*}, Anbang He\textsuperscript{a,b,c,d}, Dong Fang\textsuperscript{a,b,c,d}, Yanqing Gong\textsuperscript{a,b,c,d,\*}, Xuesong Li\textsuperscript{a,b,c,d,\*}, Liqun Zhou\textsuperscript{a,b,c,d,\*}

\textsuperscript{a} Department of Urology, Peking University First Hospital, Beijing 100034, China
\textsuperscript{b} Institute of Urology, Peking University, Beijing 100034, China
\textsuperscript{c} National Urological Cancer Center, Beijing 100034, China
\textsuperscript{d} Beijing Key Laboratory of Urogenital Diseases (male) Molecular Diagnosis and Treatment Center, Beijing 100034, China
\textsuperscript{e} Department of Urology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 45000, China

\textbf{A B S T R A C T}

\textbf{Background:} Metastatic bladder cancer (BLCA) is a lethal disease with an unmet need for study. Transgelin (TAGLN) is an actin-binding protein that affects the dynamics of the actin cytoskeleton indicating its robust potential as a metastasis initiator. Here, we sought to explore the expression pattern of TAGLN and elucidate its specific functioning and mechanisms in BLCA.

\textbf{Methods:} A comprehensive assessment of TAGLN expression in BLCA was performed in three cohorts with a total of 847 patients. The potential effects of TAGLN on BLCA were further determined using clinical genomic analyses that guided the subsequent functional and mechanistic studies. In vitro migration, invasion assays and in vivo metastatic mouse model were performed to explore the biological functions of TAGLN in BLCA cells. Immunofluorescence, western blot and correlation analysis were used to investigate the molecular mechanisms of TAGLN.

\textbf{Findings:} TAGLN was highly expressed in BLCA and correlated with advanced prognostic features. TAGLN promoted cell colony formation and cell migration and invasion both in vitro and in vivo by inducing invadopodia formation and epithelial-mesenchymal transition, during which a significant correlation between TAGLN and Slug was observed. The progression-dependent correlation between TGF-β and TAGLN was analysed at both the cellular and tissue levels, while TGF-β-mediated migration was abolished by the suppression of TAGLN.

\textbf{Interpretation:} Overall, TAGLN is a promising novel prognosis biomarker of BLCA, and its metastatic mechanisms indicate that TAGLN may represent a novel target agent that can be utilized for the clinical management of invasive and metastatic BLCA.

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1. Introduction

Bladder cancer (BLCA) is a complex disease with high morbidity and mortality rates despite improvements in its management [1]. Roughly 25% of newly diagnosed BLCA patients are diagnosed with muscle-invasive or metastatic disease. Non-muscle-invasive BLCA patients, meanwhile, continue to suffer from high recurrence (50%–70%) and progression (10%–30%) rates [2–4]. Advanced and metastatic BLCA is a lethal disease that produces poor survival outcomes. Almost all patients who succumb to BLCA have suffered from distant failure and/or symptomatic metastasis at the time of death [5]. Once cancer spreads, systemic cisplatin-based chemotherapy is the only standard treatment that can be used and usually only yields a 50% initial response rate, which results in 5-year overall and progression-free
survival of <15% [6,7]. Meanwhile, patients nearly always suffer side effects resulting from chemotherapy. Although surgical resection is also an optional treatment that can be used, only a very select group of patients with limited metastatic burden can benefit [8]. Rapid developments over the past few years have shed light on the use of immunotherapy and target therapy in the treatment of BLCA [9]. An improved understanding of invasive BLCA has emerged due to a substantial increase in molecular research that allows for the identification of the chemotherapeutic sensitivity of invasive BLCA based on its molecular subtype [10]. Increased research of invasive and metastatic BLCA will have important implications for future clinical target development and disease management.

Transgelin (TAGLN) is a well-known actin-binding protein that affects the dynamics of the actin cytoskeleton [11,12]. Owing to progress in the field of cancer biomarker discovery, studies have been conducted that TAGLN expression is decreased and dysfunctional in various cancers and that, at first, resulted in its being considered as a tumour suppressor [13]. However, subsequent studies of TAGLN and the optimization of its tissue detection produced results that were contradictory, even for the same type of tumour. Notably, its robust potential as a metastasis initiator has also been highlighted [14]. Decreased levels of TAGLN have been observed in some cancerous tissues, while increased levels have been linked with poor prognosis and metastasis, including bladder cancer. The specific expression pattern, functioning and mechanisms of TAGLN in bladder cancer remain largely unknown.

Research in context

Evidence before this study

Metastatic bladder cancer remains a complex disease with poor survival outcomes and limited treatment. Better and deeper understanding of metastatic bladder cancer is definitely needed. Transgelin (TAGLN) is a conserved cytoplasmic protein that primarily participates in remodeling of actin cytoskeleton. TAGLN was implicated in cancer development in a series of controversial studies. Decreased levels of TAGLN have been observed in some cancerous tissues, while increased levels have been linked with poor prognosis and metastasis, including bladder cancer. The specific expression pattern, functioning and mechanisms of TAGLN in bladder cancer remain largely unknown.

Added value of this study

According to our results, TAGLN was highly expressed in bladder cancer and correlated with advanced prognostic features, including stage, grade and overall survival. In line with the functional prediction, the inhibition of TAGLN repressed cell migration and invasion in vitro and led to a decrease in the number and sizes of lung metastases in vivo. Mechanistically, we found that TAGLN promotes metastasis by inducing invadopodia formation and EMT. The progression-dependent correlation between TGF-β and TAGLN was found at both the cellular and tissue levels. The higher correlation was determined for the subgroups with advanced clinicopathological features. Notably, TGF-β-mediated migration would be totally abolished by the suppression of TAGLN.

Implications of all the available evidence

Our study demonstrated that TAGLN, via its promotion of metastasis, may serve as a promising biomarker and new target agent for follow-up and treatment.
2.3. **RNA extraction and quantitative reverse-transcription PCR (RT-qPCR)**

Total RNA from tissues or cells was extracted using TRIzol reagent (Invitrogen, USA) and reverse-transcribed using a Fastking RT-PCR kit (Tiangen, China) according to the manufacturer's instructions. Quantitative PCR was conducted using SYBR Green Premix reagent (Tiangen, China) and an ABI PRISM 7000 Fluorescent Quantitative PCR System (Applied Biosystems, USA) according to the manufacturer's instructions. The sequences of the primers used in this study are listed in Supplementary Table S3. The data analysis was performed using the ΔΔCt method, and expression was normalized to that of β-actin.

2.4. **In silico analysis of TAGLN using online datasets**

A transcriptome dataset obtained from BLCA tissues (TCGA-BLCA) from the Cancer Genome Atlas (TCGA) was downloaded using the UCSC Xena browser in June 2018; data from the GSE13507-BLCA cohort was obtained from the gene expression omnibus (GEO) dataset GSE13507. The patients in each cohort were sorted into high and low groups based on the median of TAGLN expression level. Differentially expressed genes (DEGs) were selected using the TwoClassDiff method [25,26]. All enrichment analyses were performed using Metascape (http://metascape.org) [27]. The assessment and integration of protein–protein interactions were conducted using the STRING database [28] and modelled using Cytoscape [29]. The minimum-required interaction score generated using STRING was set to that corresponding to a medium confidence level (0.400). The IHC images in Supplementary Fig. S4b were obtained from Human Protein Atlas (www.proteinatlas.org) [30]. The prognostic analyses shown in Supplementary Fig. S4c–h were obtained from GEPIA (http://gepia.cancer-pku.cn/index.html) [31].

2.5. **Cell lines and cell culture**

Authenticated SV-HUC-1, SW780, 5637, T24, and 293 T cells were obtained from the American Type Culture Collection (ATCC). The SV-HUC-1 cells were cultured in F-12 K (Gibco, USA), the SW780, T24 and 293 T cells were cultured in DMEM (Gibco, USA) and the 5637 cells were cultured in RPMI 1640 (Gibco, USA). All media were supplemented with 10% foetal bovine serum and 1% penicillin-streptomycin. All cells were cultured in a humified atmosphere containing 5% CO2 at 37 °C and were maintained in low passage cultures throughout the experiments.

2.6. **Western blotting**

The total cellular protein was extracted using RIPA (Millipore, USA) and 1 mM phenylmethylsulfonyl fluoride (PMSF) and was quantified via a bichinchoninic acid assay (Sigma, USA). The lysates were then separated using SDS–PAGE and electroblotted onto polyvinylidene difluoride membranes. After incubation with the primary and secondary antibodies in sequence, the bands were visualized via enhanced chemiluminescence using an ImmobilonTM Western Kit (Millipore, USA). β-actin served as a loading control.

2.7. **Immunofluorescence assay**

Cells were cultured on glass coverslips for 24 h and subsequently fixed with 4% paraformaldehyde for 30 min at room temperature and permeabilized with 0.2% Triton. The coverslips were then probed with the primary antibody and the corresponding fluorescent secondary antibody. The labelled cells were then stained with 4′,6-diamidino-2-phenylindole (DAPI) and mounted using antifade mounting medium. Phalloidin-tetramethylrhodamine B isothiocyanate (Sigma, USA) was used to label actin. All slides were examined using multiphoton lifetime confocal laser-scanning microscopy (TCS SP8; Leica, Germany).

2.8. **Cell transfection**

The plasmids used in this study were purchased from Beijing Syngentech. The shRNA sequences that were used are shown in Supplementary Table S3. The plasmids of interest and the packaging plasmids into were co-transfected into 293 T cells for the purposes of lentiviral production, and subsequently the viral supernatants were titrated using RT-qPCR for which the WPRE (woodchuck hepatitis virus posttranscriptional regulatory element) was used as the primer. All BLCA cells were infected with lentivirus at a MOI (multiplicity of infection) of 10. The stable cells were selected using puromycin.

2.9. **Cell viability, cell cycle, and cell colony formation assays**

The cell viability assays were performed using the CellTiter 96® AQ One Solution Reagent (Promega, USA). The cell cycle assays were performed using a Cell Cycle Detection Kit (KeyGEN BioTECH, China). All procedures were performed according to the manufacturer's instructions. For the cell colony formation assay, 1000 cells/well were seeded in a 6-well plate and cultured for 10 days. The colonies were then fixed and stained with a 0.5% crystal violet methanol solution for 30 min and scanned to generate images.

2.10. **Cell migration and invasion assays**

Cell migration was first assessed via wound healing assays. After seeding the cells in a 6-well plate for 24 h, straight vertical and horizontal lines were scratched using a 200 μl pipette tip. Images were obtained using the digital camera that was attached to the microscope immediately after the scratches were made and 48 h later. Cell migration was also measured via transwell migration assays using chambers with membranes containing 8 μm pores (Corning, USA). The cells were suspended in 100 μl of serum-free media and seeded in the upper chamber, and the lower chamber was filled with 500 μl of complete media. After incubation at 37 °C for 24 h, the transwell membranes were fixed and stained with 0.5% crystal violet methanol solution for 30 min. The un-migrated cells were removed using cotton swabs and photographed with the digital camera. The cell invasion assays were performed similarly to the transwell migration assays with transwell membranes that were coated with Matrigel (BD Biosciences) and were incubated for 48 h.

2.11. **Tail vein injection metastatic mouse model**

All animal procedures were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals with the approval of the Institutional Animal Care and Use Committee of Peking University First Hospital. T24-Luc cells, which are T24 cells that stably express luciferase, were used in this model. One hundred microliters of cell suspension (2 × 10^7/ml) was injected into the lateral tail vein of each 6-week-old B-NDG mouse (Biocytogen, China). Six weeks after injection, the mice were anesthetized with isoflurane (Yipin Pharmaceutical, China), and then D-luciferin sodium salt (Biovision, USA) was injected intraperitoneally according to the manufacturer's instructions. The metastatic lesions were visualized using a Xenogen IVIS system (PerkinElmer, USA) and were then harvested and prepared for H&E staining and IHC.

2.12. **Statistical analysis**

All experiments were performed independently at least three times except for the experiments shown in Figs. 1–2, 3f–g, 4e, 5a–f,
Fig. 1. TAGLN overexpression correlates with the presence of poor prognostic features in BLCA. a. Representative immunohistochemical staining images of TAGLN protein in cancer tissues and paired normal tissues. P1–P4 were four representative patients with different clinicopathological features. Scale bar, 50 μm. b. Percentage distribution of TAGLN IHC intensity in cancer versus normal tissues. The staining intensity was graded as – (negative), + (weak), ++ (moderate) or +++ (strong). c. Percentage distribution of TAGLN IHC intensity in BLCA with various clinicopathological features. POM, postoperative cancer metastasis. d. Kaplan-Meier curves of the TAGLN IHC intensity on OS in PKU-BLCA cohort. e. Kaplan-Meier curves of the TAGLN IHC intensity on MFS in PKU-BLCA cohort. f. General prognostic signatures and meta-analyses of TAGLN in three cohort. All of the prognostic events of TAGLN are illustrated in forest plots, with corresponding P value and 95% CI. The estimated effect size of each event is presented as a black square that is proportional in size to the weight of the study. The pooled effect size is presented as a red rhombus that is sized in the center for summary effect size and whose width depicts the confidence interval. The confidence interval of effect size appears as a horizontal. The n values indicate the number of patients. *P < .05; **P < .01.
The cut-off values used for TAGLN in Supplementary Fig. S1f and S1g were defined using X title [32]. Univariate survival analyses were performed by Kaplan-Meier estimator (Log-rank test) and multivariate analyses used backward method (Wald test). All meta-analyses were performed with Stata12.0 (StataCorp LP, USA). All statistical tests were performed using SPSS software version 22.0 (SPSS, USA). A P value <.05 was considered to be statistically significant.
3. Results

3.1. TAGLN overexpression correlates with the presence of poor prognostic features in BLCA

To validate the differences in the levels of TAGLN transcription in BLCA and normal tissue, TAGLN mRNA was measured using RT-qPCR in 27 paired frozen tissues. Consistent with the results of previous studies [21,22], TAGLN mRNA was downregulated in the BLCA tissues (Supplementary Fig. S1a). A similar result was also observed in both the TCGA-BLCA and GSE13507-BLCA cohorts (Supplementary Fig. S1b-c). To further verify the expression of TAGLN in BLCA and normal tissues, 104 paired paraffin-embedded tissue sections were examined using immunohistochemistry (IHC) staining. Unlike the RT-qPCR results, the IHC results showed that TAGLN was significantly upregulated in BLCA tissues compared with normal bladder tissues (Fig. 1a-b). Consistent with previous findings, TAGLN was mainly localized in the cytosol in both normal and cancerous tissues [14].

To confirm the relationship between TAGLN and BLCA, we next evaluated the correlation between the TAGLN expression level and clinicopathological features in three cohorts (Supplementary Table S1). In the PKU-BLCA cohort, statistical analysis of a total of 275 BLCA patients revealed that the TAGLN protein level was correlated with the pathological T, pathologic N, pathologic stage and histologic grade (Fig. 1c, Table 1). Notably, higher TAGLN expression level contributed to higher rate of postoperative cancer metastasis. These results were confirmed in the other cohorts. The TAGLN mRNA level was significantly correlated with the pathologic T, pathologic N, pathologic stage and histologic grade in both the TCGA-BLCA and GSE13507-BLCA cohorts (Supplementary Fig. S1d-e). There was no significant correlation between TAGLN expression and the pathologic M due to the limited number of M1 patients in the cohorts. However, the number of patients with high TAGLN expression was twice as high as the number with low TAGLN expression in both two pathologic M1 cohorts (Supplementary Table S4). Furthermore, both univariate and multivariate analyses showed that patients with BLCA that expressed high levels of TAGLN had a shorter overall survival (OS) than patients with cancers that expressed low levels of TAGLN in all three cohorts (Fig. 1d, Table 2; Supplementary Fig. S1f-g, Supplementary Table S5). Crucially, we also found that high TAGLN level was significantly correlated with poor metastasis-free survival (MFS) of BLCA patients in PKU-BLCA cohort (Fig. 1e).

To summarize the correlation between the TAGLN expression level and clinicopathological features in all three cohorts, meta-analyses were performed for each feature (Fig. 1f). Based on these meta-analyses, TAGLN, which showed high consistency for all features, was significantly associated with unfavourable prognosis for all four clinical features (T, OR, odds ratios: 3.827; N, OR: 2.997; stage, OR: 4.053; grade, OR: 2.901; P < .0001) and one survival feature (OS, HR, hazard ratio: 2.25; P < .001). All of the results demonstrated that increased TAGLN expression contributes to poor prognosis in BLCA patients, which suggests a potential link between TAGLN and BLCA progression.

3.2. Functional prediction of TAGLN in silico analysis

In order to reveal the potential function of TAGLN in BLCA, the patients were separated into low- and high- TAGLN expression groups based on the median expression level in the TCGA and GSE13507 cohorts, respectively. The differentially expressed genes (DEGs) in the two cohorts were determined (Fig. 2a, Supplementary Table S6-1). Additional enrichment analyses were performed using the consensus upregulated genes in the high TAGLN group. EMT-related genes were significantly highlighted during the cancer hallmark enrichment analysis (Fig. 2b, Supplementary Table S6-2). KEGG pathway and GO term enrichment analyses were also performed. Genes involved in focal adhesion, leucocyte trans-endothelial migration, regulation of the actin cytoskeleton and relevant member pathways were enriched (Fig. 2c, Supplementary Table S6-3). Terms related to extracellular matrix organization, supramolecular fibre organization, the positive regulation of cell motility and relevant member terms were also enriched (Fig. 2d, Supplementary Table S6-4). All enrichment analyses indicated that TAGLN is involved in actin cytoskeleton regulation, cell adhesion, cell motility and EMT in BLCA.

Actin is a ubiquitous and essential protein involved in tumour cell motility, structure and integrity that is also involved in the formation, stabilization and maturation of invadopodia in cancer [33,34]. Additionally, the role of TAGLN in the processes of cell migration and metastasis has been described in both smooth muscle cells and cancer cells, in which it contributes to the formation of podosomes [35–37]. Thus, we speculated that TAGLN may participate in invadopodia formation. Additional verification analyses were performed in the TCGA-BLCA cohort. Based on the Spearman correlation coefficient, both the EMT- and invadopodium-associated genes showed a significant correlation with TAGLN (Fig. 2e). The expression level of these genes also showed significant differences in the low- and high-TAGLN expression groups, which confirms the potential relationship of TAGLN with EMT and invadopodia (Fig. 2f-g). Next, the potential interaction targets of TAGLN in the protein-protein interaction network were determined (Fig. 2h), including actin-associated genes (ACTA2, ACTB, ACTG2) and EMT-associated genes (TFGB1, MMP9, COL1A1). Overall, all these results indicated that TAGLN may be involved in cell migration and metastasis resulting from invadopodia formation and EMT in BLCA.

3.3. TAGLN promotes the clonogenicity of BLCA cells

To validate the predicted function of TAGLN, we performed a series of functional assays using the relevant cell lines. The expression levels of TAGLN in BLCA cells (SW780, 5637 and T24) and a normal uroepithelium cell line (SV-HUC-1) were examined using RT-qPCR and western blotting (Fig. 3a). Compared with SV-HUC-1, TAGLN was upregulated in T24 cells and downregulated in 5637 cells. BLCA cell lines were then established that stably overexpressed either TAGLN or an shRNA that targeted TAGLN (Fig. 3b). To assess the effects of TAGLN on proliferation, cell viability and cell cycle assays were performed on the T24-shTAGLN and 5637-TAGLN cells. Both assays showed that TAGLN expression had essentially no effect on cell growth or the distribution of cells in the cell cycle (Supplementary Fig. S2a-b). Interestingly, there were significant changes in colony formation (Fig. 3c). Both the number and size of the colonies were affected by the expression level of TAGLN. These results demonstrated that TAGLN was involved in the clonogenicity of BLCA cells rather than cell proliferation.
Fig. 4. TAGLN promotes invadopodia formation and EMT. a. Representative anti-TAGLN immunofluorescence images of TAGLN-silenced cells and TAGLN-overexpressed cells. Cells were counterstained with DAPI; combined images were indicated. Scale bar, 10 μm. b. Representative invadopodia immunofluorescence images in BLCA cells. TAGLN-silenced cells, TAGLN-overexpressed cells and control cells stained for nuclei (blue), F-actin (red), and CTTN (green). Quantification of the percentage of cells with invadopodia. N = 50 cells/sample. Scale bars, 10 μm in T24 (a); 5 μm in 5637 (b). c-d. Western blot and RT-qPCR analyses of EMT marker and transcription factors after TAGLN depletion in T24 (c) and TAGLN overexpression in SW780 and 5637 (d). e. Representative IHC staining images of TAGLN and Slug in lung metastatic of tail vein-injected T24-Luc metastasis model. Scale bars, 50 μm. Error bars represent SD of the mean. *, P < .05; **, P < .01.
3.4. TAGLN promotes the migration and invasion of BLCA cells

To characterize the role of TAGLN in cell migration, a wound healing assay was performed in T24-shTAGLN and 5637-TAGLN cells. Their migratory capabilities were significantly decreased in T24-shTAGLN cells and increased in 5637-TAGLN cells (Fig. 3d). These results were also confirmed using transwell migration assays (Fig. 3e). Similarly, invasive capability was dramatically reduced in TAGLN-silenced cells and enhanced in TAGLN-overexpressed cells (Fig. 3e). To confirm the positive effects of TAGLN on cell metastasis, a tail-vein injection metastasis model was established using luciferase-labelled T24 (T24-Luc). A significant reduction in tumour metastasis in the TAGLN-silenced group compared with the control group was observed (Fig. 3f). Based on HE and IHC staining, the number and sizes of the metastatic lesions in the TAGLN-silenced group were significantly decreased (Fig. 3g). Overall, these results indicated that TAGLN promotes the invasiveness of BLCA cells both in vitro and in vivo, which is consistent with the earlier functional prediction.

3.5. TAGLN promotes invadopodia formation and EMT

It is well-known that tumour cell invadopodia play a vital role in tumour metastasis [38]. Notably, TAGLN may promote cell motility by regulating the formation of invadopodia, which is in line with the results of the functional prediction analyses. Invadopodia are actin-based dynamic protrusions that utilize the actin regulators cortactin, N-WASP, TKS4, TKS5, and MMP14 [39]. Thus, we evaluated the correlation between the presence of invadopodia and the co-localization of F-actin with cortactin (CTTN) in bladder cell lines. Based on immunofluorescence staining, both the up- and downregulated TAGLN was distributed primarily in the cytoplasm, which indicates its association with actin in BLCA (Fig. 4a). Also, we found that the cell lines with greater expression of TAGLN also showed higher expression of cortactin (Supplementary Fig. S2c–d). In T24 cells, which exhibited a high metastatic capability and a high level of TAGLN, over 75% of the cells contained invadopodia, while only 40–60% of 5637 or SW780 cells, both of which have a low metastatic capability and lower level of TAGLN, contained invadopodia (Supplementary Fig. S2e). Next, we examined the effects of TAGLN on...
invadopodia formation. Suppression of TAGLN significantly reduced the number of invadopodia in T24-shTAGLN (approximately 40% of cells) and SW780-shTAGLN (30% of cells) cells. Meanwhile, the number of cells containing invadopodia was increased to 80% in the TAGLN-overexpressed cell line 5637-TAGLN, and the invadopodia contained a greater number of puncta as well as larger puncta (Fig. 4b). These results strongly demonstrate that TAGLN is involved in invadopodia formation.

Reactivation of EMT in cancer cells enhances the metastatic phenotype [40]. As suggested by the functional prediction as well as the positive effect of TAGLN on cancer metastasis, there was a potential reliable connection between TAGLN and EMT. EMT markers were detected in both the TAGLN-silenced and TAGLN-overexpressed cells. The expression of the mesenchymal markers/transcription factors N-cadherin, β-catenin and Slug were significantly decreased in TAGLN-silenced cells and increased in TAGLN-transduced cells, while the expression of the epithelial marker E-cadherin was downregulated in TAGLN-overexpressed cells (Fig. 4c-d). At the same time, the expression level of MMP14, which is also involved in the EMT process [40] and invadopodia formation [41], was decreased via the downregulation of TAGLN and increased via the upregulation of TAGLN. These results were also confirmed using RT-qPCR. We also further confirmed the correlation between TAGLN and Slug expression in the metastatic animal model. The expression level of Slug, which is distributed primarily in the nucleus, was dramatically decreased by the inhibition of TAGLN (Fig. 4e). All of these results were also highly consistent with the earlier functional prediction analysis, which demonstrated that TAGLN promotes metastasis by inducing invadopodia formation and EMT.

### 3.6. TAGLN is essential for the promotion of BLCA metastasis by TGF-β

The TGF-β signalling pathway is one of the most well-characterized and vital pathways involved in the induction of cancer metastasis and EMT [42]. Based on the involvement of TAGLN in cancer metastasis and EMT in BLCA cells and the regulation of TAGLN by TGF-β that was observed in previous studies [43,44], we further analysed the expression patterns of TGF-β and TAGLN at both the tissue and cellular levels. In the 26 BLCA cell lines included in the CCLE (Supplementary Fig. S3a), a significant positive correlation between TGFβ2 and TAGLN was found (Spearman’s correlation r = 0.556, P < .01). Based on the clinicopathological features of each cell line [45], correlation tests were performed in each of the various subgroups. Significant correlations between TGFβ2 and TAGLN were only found in relation to advanced clinicopathological features, such as advanced T and G (Fig. 5a, Supplementary Fig. S3b–c). Our results also showed that the expression of TGFβ2 and TAGLN was closely associated in basal subtype BLCA cells, which were more aggressive and resulted in reduced survival compared with luminal cancers (Fig. 5d) [10,46]. To confirm the correlation between TGF-β and TAGLN at the tissue level, similar analyses were performed in the TCGA-BLCA cohort and the GSE13507-BLCA cohort. Interestingly, TAGLN was more closely correlated with TGFβ3 than TGFβ2 and TGFβ1 in BLCA tissues (Supplementary Fig. S3d–e). In line with the cell analyses, higher correlation coefficients were determined for the subgroups with advanced clinicopathological features (Fig. 5b–c,e–f, Supplementary Fig. S3f–n). In particular, high correlation coefficients were obtained for the pathologic M1 subgroups in both cohorts. Additionally, analysis of 23 primary- recurrent paired BLCA tissues from the GSE13507-BLCA cohort indicated that a significant positive correlation between TGFβ3 and TAGLN existed only in the recurrent group rather than the primary tumour group. These results provide support for the presence of a tight functional correlation between TGF-β and TAGLN during tumour development and metastasis in BLCA.

Given the strong association between TGF-β and TAGLN that is observed during the progression of BLCA, confirmatory tests were performed in BLCA cell lines. The expression levels of TAGLN and mesenchymal markers/transcription factors were measured in the presence of TGF-β3. As expected, the TAGLN and mesenchymal markers/manifestations of TGF-

### Table 1

**Correlation between TAGLN immunostaining intensity and clinicopathological features in PKU-BLCA cohort.**

| Cohort characteristics | TAGLN immunostaining intensity | P value |
|------------------------|--------------------------------|---------|
|                        | – | + | ++ | +++ | ++++ |
| **Gender**             | Female | 12 | 12 | 9 | 6 | 0.9221 |
|                        | Male   | 69 | 63 | 64 | 40 |         |
| **Age (median 67, year)** | ≤67 | 48 | 43 | 30 | 25 | 0.1103 |
|                        | >67    | 33 | 32 | 43 | 21 |         |
| **Pathologic T**       | T1 | 45 | 30 | 10 | 6 | -0.0001 |
|                        | T2    | 27 | 27 | 21 | 7 |         |
|                        | T3    | 5  | 12 | 15 | 18 |         |
|                        | T4    | 4  | 12 | 21 | 11 |         |
| **Pathologic N**       | N0    | 73 | 55 | 52 | 28 | 0.0146 |
|                        | N1    | 3  | 7  | 5  | 4  |         |
|                        | N2    | 3  | 6  | 10 | 10 |         |
|                        | N3    | 0  | 0  | 0  | 1  |         |
| **Pathologic stage**   | I     | 44 | 28 | 8  | 5  | -0.0001 |
|                        | II    | 27 | 19 | 24 | 9  |         |
|                        | III   | 4  | 14 | 23 | 18 |         |
|                        | IV    | 6  | 14 | 18 | 14 |         |
| **Histologic grade**   | G1–2  | 32 | 23 | 13 | 6  | 0.0021 |
|                        | G3    | 49 | 52 | 40 | 60 |         |
| **Postoperative cancer metastasis** | Absent | 33 | 28 | 25 | 13 | -0.0001 |
|                        | Present| 16 | 14 | 30 | 30 |         |

### Table 2

Univariate and multivariate analyses of clinicopathological characteristics and TAGLN expression with overall survival in PKU-BLCA cohort.

| Cohort characteristics | Univariate analysis* | P value | Multivariate analysis* | P value |
|------------------------|----------------------|---------|------------------------|---------|
|                        | HR(95% CI)            |         | HR(95% CI)             |         |
| **TAGLN**              |                      |         |                        |         |
| +/+ +/+ +/+ +/+ vs. –  | 3.02(1.86–4.903)     | 0.001   | 2.477(1.016–6.041)     | 0.046   |
| Female vs. Male         | 1.171(0.602–2.28)    | 0.642   |                        |         |
| Age                    | –median vs. smedian  | 1.654(1.046–2.616) | 0.031 |                        |         |
| **Pathologic T**        |                      |         |                        |         |
| T3–4 vs. Tis-2         | 3.534(2.241–5.637)   | <0.001  | 2.158(1.18–3.948)      | 0.013   |
| **Pathologic N**        |                      |         |                        |         |
| N1–3 vs. N0            | 3.632(2.022–5.991)   | <0.001  | 2.396(1.307–4.393)     | 0.005   |
| **Histologic grade**   |                      |         |                        |         |
| High grade vs. Low grade | 2.172(1.171–4.03)   | 0.014   |                        |         |
| Postoperative chemotherapy Yes vs. No | 2.194(1.283–3.753) | 0.004  |                        |         |

* Univariate analysis were performed by Kaplan-Meier estimator (Log-rank test).
  Multivariate analysis used backward method (Wald test).
transcription factors expression levels were upregulated by TGF-β stimulation (Fig. 5g). Notably, the stimulating effect of TGF-β was attenuated by MMP14 and Slug in the TAGLN-silenced group (Fig. 5g). Functionally, the migratory ability of BLCA cells was dramatically increased after TGF-β stimulation. However, TGF-β-induced migration was completely abolished by the inhibition of TAGLN, especially in T24-shTAGLN#2 cells. Meanwhile, the effects of TAGLN silencing on migration was slightly rescued by TGF-β in T24-shTAGLN#1 cells, which indicates that the migratory ability of BLCA cells depends greatly on the TAGLN expression level (Fig. 5h). Overall, the results show that TAGLN is essential for TGF-β-induced metastasis in BLCA.

4. Discussion

In this study, we explored the expression patterns and molecular function of TAGLN in BLCA using specimen detection analysis, bioinformatics analysis and a series of functional assays both in vitro and in vivo. The results demonstrated that TAGLN contributes to poor prognosis in BLCA. TAGLN overexpression may promote the migration and invasion of BLCA cells via invadopodia formation and the induction of EMT. The strong relationship between TGF-β and TAGLN was demonstrated during the progression of BLCA. Additionally, TGF-β-mediated migration was shown to be abolished by the inhibition of TAGLN. These results provide further insight regarding the metastasis of BLCA, which implies that TAGLN may serve as a potential treatment target for BLCA metastasis.

Based on immunohistochemical staining of 104 paired BLCA tissues, TAGLN is upregulated in cancer tissues. However, our RT-qPCR results from 27 paired BLCA tissues as well as an online dataset and previous studies [21–22] have indicated that the TAGLN mRNA expression level is decreased in BLCA tissues compared with normal bladder tissues. It is important to note that all of the normal bladder tissues that were used in this study were complex tissues that were not subject to microdissection and consisted not only of bladder epithelial cell but also stromal cells and smooth muscle cells (SMCs). It is well known that TAGLN is an abundant protein in SMCs [47,48]. According to the IHC results obtained from the PKU-BLCA cohort, SMCs were found in almost all of the normal bladder samples (Supplementary Fig.S4a). This was also confirmed using the online database Human Protein Atlas (Supplementary Fig. S4b). Although the BLCA tissues also consist SMCs, especially muscle-invasive BLCA, the limited bladder epithelium might more easily be distorted by the unmeasured smooth muscle cells comparing to the bladder cancer tissue. Ambiguous results, such as those that have been found for TAGLN, have also been found for other cancers that affect SMC-rich tissues, including oesophageal cancer [15,16] and colorectal cancer [19,20]. Therefore, it is important to consider the rationale design of the experiments, such as applying tissues microdissection and multomics analysis to the validation. Upon the integration of clinicopathological features, the contribution of TAGLN to poor prognosis has been observed in all three cohorts. As a benefit of the meta-analyses, the combined effects of each prognostic feature were determined in all three cohorts, which all demonstrated the unfavourable effects of TAGLN on BLCA. Collectively, the overall unfavourable expression pattern of TAGLN in BLCA indicates that TAGLN plays an important role in the development and progression of BLCA. Additionally, aberrant expression of TAGLN has also been shown to be involved in other cancers; for example, unfavourable prognostic effects have been noted for colorectal cancer. TAGLN upregulation has been correlated with submucosal invasiveness [49], lymph node invasiveness [19] and poor prognosis [50] in colorectal cancer. According to prognostic analysis of the TCGA dataset, the unfavourable effects of TAGLN on overall survival have been noted for adrenocortical carcinoma, kidney cancer, lower grade gliomas, squamous cell carcinoma of the lung, mesothelioma and uveal melanoma (Supplementary Fig. S4c–h). In contrast, TAGLN has been shown to be decreased in lymph node metastatic prostate cancer [51] and triple-negative breast cancer [52], which indicates the favourable prognostic effects of TAGLN. Differences in the cancer of origin may account for the various effects of TAGLN; for example, the bladder and colorectum are tract organs and of endodermic origin, while the prostate and breast are sex hormone-targeted glands. It is important to elucidate the role of TAGLN in each type of cancer separately.

To better understand the oncogenic role of TAGLN in BLCA, step–forward functional prediction and verification were performed. As indicated by the enrichment analyses, TAGLN may be involved in actin regulation and cell motility in BLCA. Actin serves as a key player in cell migration [33]. As an actin-binding protein, TAGLN shows great potential in altering motility via its interactions with actin. The verification assays proved that TAGLN promotes the migration and invasion of BLCA cells both in vitro and in vivo. Interestingly, TAGLN promotes the clonogenicity of BLCA cells rather than cell proliferation, which indicates the potential role of TAGLN in metastatic colonization. All of these functions of TAGLN in BLCA were correlated with the above-mentioned unfavourable prognostic effects of TAGLN. It was demonstrated that TAGLN levels significantly affect metastatic potential. To explain the role of TAGLN in BLCA metastasis more clearly, the relationships between TAGLN, EMT and invadopodia were documented. First, the involvement of TAGLN in EMT was highlighted in the cancer hallmark enrichment analysis. Our study demonstrated that the TAGLN expression level was significantly associated with EMT markers in terms of both perdition and verification. EMT status affects the metastatic potential [40]. During EMT in myofibroblasts (EMyf1), the transcription factor serum response factor (SRF) and myocardin family transcription factors (MRTFs) are able to activate TAGLN transcription [53]. TAGLN is normally expressed in mesenchymal cells [48], especially in smooth muscle cells, and serves as an early marker of smooth muscle differentiation [54]; its appearance in epithelial-derived tumour cells is related to EMT, whereby tumour cells acquire greater metastatic potential. Indeed, the BLCA cell line T24, which is characterized by mesenchymal tumour cells that express extremely low levels of E-cadherin level and have elongated shapes, also expresses a high level of TAGLN and exhibits an aggressive metastatic potential in comparison to the epithelial tumour cell line 5637. Thus, TAGLN may serve as a type of EMT indicator in BLCA. Notably, we found that the TAGLN expression level also affect EMTs. The expression of mesenchymal state markers and transcription factors was decreased after the inhibition of TAGLN and were increased by the overexpression of TAGLN. Likewise, an EMT-regulatory function of TAGLN was identified during a previous colorectal cancer study [19]. Therefore, the metastatic modulation of TAGLN should influence EMT. Next, we also showed that TAGLN participates in invadopodia formation, which supports the role of TAGLN in the promotion of metastasis. During podosome formation in SMCs, TAGLN is recruited and TAGLN-decorated actin bundles undergo rapid reorganization into podosomes [36,37]. Since podosomes and invadopodia are highly similar and nearly indistinguishable, we applied rules that state that ‘normal cells have podosomes’ and ‘cancer cells have invadopodia’ [39]. Previous studies as well as our prediction analyses have revealed the strong interaction of actin and TAGLN. Actin dynamics are a key element of the assembly and maintenance of invadopodia [34]. Due to the integration of the involvement of TAGLN in podosomes with the vital role of actin in invadopodia formation, we hypothesized that TAGLN also plays an important role in invadopodia formation in BLCA. The positive correlation between TAGLN and invadopodia–associated genes in BLCA was documented during the prediction analysis. Our results also showed that a reduction in TAGLN inhibits invadopodia formation, while invadopodia formation is promoted by TAGLN overexpression in BLCA cells. Invadopodium maturation results from matrix metalloproteinase (MMP) recruitment, which involves the delivery of MMP14 (MT1-MMP) to invadopodia and the activation of local matrix degradation [41]. In this context, MMP14 was significantly reduced by the depletion of TAGLN and increased by TAGLN overexpression in BLCA cells. TAGLN was shown to play specific roles in invadopodia formation in BLCA. The association between EMT and in invadopodia formation appears to be
inseparable during the systemic spread of tumours [55,56]. Overall, the involvement of TAGLN in EMT and invadopodia formation contribute to its robust metastatic potential. Nevertheless, the precise involvement of TAGLN in EMT and invadopodia formation will require further evaluation.

The question of the factors involved in the regulation of the expression of TAGLN during the progression of BLCA was raised. Serum response factor (SRF)–binding CArG boxes, a Smad–binding element (SBE) and a TGF-β control element (TCE) are included in the TAGLN promoter [43,44], which suggests the vital roles played by SRF and TGF-β in TAGLN regulation. Although many studies have shown that the TGF-β signalling pathway regulates TAGLN expression in various systems [57–59], this regulation is rarely studied in cancer. TGF-β signalling is undoubtedly a central player in cancer progression due to its dual functional role [42]. Not to mention, TGF-β acts as a lymph node metastatic biomarker [60] and is involved in the invasion and metastasis of BLCA [61,62]. Furthermore, TGF-β reprograms the tumour microenvironment to attenuate anti-tumour immunity in metastatic bladder cancer [63]. Therefore, we analysed the expression patterns of TGF-β and TAGLN in both cells (26 cell lines) and tissues (572 patients) in BLCA. As expected, a tight positive correlation between TGF-β and TAGLN was found in BLCA. Notably, only subgroups with advanced clinicopathological features reflected a significant relationship via increased correlation coefficients, which implies that TGF-β promotes TAGLN expression during cancer progression. TGF-β signalling induces EMT [42] and promotes invadopodia formation [55,56]. This supports the previous assumption that TAGLN promotes metastasis via EMT and invadopodia formation. Additionally, the TGF-β–stimulated upregulation of Slug and MMP14 were attenuated by TAGLN inhibition, while cell migration induced by TGF-β was totally abolished, which indicated the essential role of TAGLN in the promotion of TGF-β of BLCA metastasis. Collectively, TAGLN may serve as a powerful treatment target for TGF-β-mediated metastasis originating from BLCA.

Although there is no selective TAGLN inhibitor yet, quite a few drugs show available potential. Since the progression-dependent correlation between TGF-β and TAGLN was highlighted in bladder cancer, TGF-β inhibitors could serve as potential drugs. Virtually, several TGF-β pathway targeting drugs have been applied into clinical trials and many more have been tested through numerous design strategies [64]. SB-431542, a TGF-β/Smad3 inhibitor in preclinical development, could effective downregulate TAGLN expression level indicating its promising application potential in clinic [58]. Recently, Zhong et al. reported that salvianolic acid A enhances vasoconstriction by targeting the transgelin-actin complex [65]. Salvianolic acid, a well-known treatment compound for cardiovascular-related diseases, was discovered as novel and effective drugs for cancer treatment by promoting cells apoptosis and inhibiting EMT transistion [66]. TAGLN2 (transgelin-2), one of TAGLN’s homologs with 64% amino acid sequence homology to TAGLN [14], was supressed by salvianolic acid A resulting in inhibition of breast cancer cells migration and invasion [67]. Taken together, these perspectives on the correlation between salvianolic acid A and TAGLN highlight the robust therapeutic potential of salvianolic acid A as TAGLN-targeting drug. However, all the therapeutic potential still need being well studied in order to be applied to the treatment.

In summary, TAGLN has been identified as a novel prognosis predictor due to its unfavourable prognostic effects and its function as a metastatic driver via EMT and invadopodia formation and was also shown to be induced by TGF-β in BLCA. Future studies of the specific roles played by TAGLN in EMT and invadopodia formation are critical to increase our knowledge of BLCA metastasis. TAGLN, via its promotion of metastasis, may serve as a promising biomarker and new target agent for follow-up and treatment.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebiom.2019.08.012.

Data accessibility

The transcriptome dataset of bladder cancer from The Cancer Genome Atlas (TCGA), TCGA–BLCA cohort, was downloaded from UCSC Xena browser in June 2018, while GSE13507–BLCA cohort was obtained from gene expression omnibus (GEO) dataset: GSE13507. The other data generated or analysed during this study are included in this published article and its Additional file information files.

Authors’ contributions

Conception and design: Z.C, S.H, Y.G, X.L, and L.Z; Methodology development: Z.C, S.H, and Y.Z; Acquisition of clinical specimens and information: Z.C, Y.Z, A.H, and D.F; Experiment implementation: Z.C and Y.Z; Analysis and interpretation of data: Z.C and S.H; Drafting of the manuscript: Z.C; Review and revision of the manuscript: Y.G, S.H, X.L, and L.Z; Administrative support, obtaining funding, supervision: S.H, Y.G, X. L, and L.Z. All authors read and approved the submitted manuscript.

Declaration of Competing Interest

All authors declare that there are no conflicts of interest.

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References

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68(6):394–424.
[2] Li K, Liu T, Chinese Bladder Cancer C, Xue W, Mu X, Xu E, et al. Current status of diagnosis and treatment of bladder cancer in China - analyses of Chinese bladder cancer consortium database. Asian J Urol 2015;2(2):63–9.
[3] Comperat E, Larre S, Roupret M, Neuzillet Y, Pignot G, Quinetns H, et al. Clinicopathological characteristics of urothelial bladder cancer in patients less than 40 years old. Virchows Arch 2015;466(5):589–94.
[4] Czubmiett S, Sylveostore RJ, Collerte L, Gontore P, Brausi MA, Van Andel G, et al. EORTC nomograms and risk groups for predicting recurrence, progression, and disease-specific and overall survival in non-muscle-invasive stage Ta-T1 urothelial bladder cancer patients treated with 1–3 years of maintenance bacillus calmette-guérin. Eur Urol 2016;69(1):60–9.
[5] Abufaraj M, Gust K, Moschini M, Foerster B, Soria F, Mathieu R, et al. Management of muscle invasive, locally advanced and metastatic urothelial carcinoma of the bladder: a literature review with emphasis on the role of surgery. Transl Androl Urol 2016;5(3):735–44.
[6] von der Maase H, Hansen SW, Roberts JT, Dogliotti L, Oliver T, Moore MJ, et al. Gemcitabine and cisplatin versus methotrexate, vinblastine, doxorubicin, and cisplatin in advanced or metastatic bladder cancer: results of a large, randomized, multination, multicenter, phase III study. J Clin Oncol 2000;18(17):3068–77.
[7] von der Maase H, Sengelov L, Roberts JT, Ricci S, Dogliotti L, Oliver T, et al. Long-term survival results of a randomized trial comparing gemcitabine plus cisplatin, with methotrexate, vinblastine, doxorubicin, plus cisplatin in patients with bladder cancer. J Clin Oncol 2005;23(21):4602–8.
[8] Abuducar M, Dalbagost G, Daneshmand S, Horenblad S, Kamat AM, Kanzaki R, et al. The role of surgery in metastatic bladder cancer: a systematic review. Eur Urol 2018;73 (4):543–57.
[9] Kamat AM, Hahn NM, Efstatthiou JA, Lerner SP, Mallminstrum P-U, Choi W, et al. Bladder cancer. The Lancet 2016;388(10061):P2796–8.
[10] Choi W, Porten S, Kim S, Willis D, Plimack ER, Hoffman-Censits J, et al. Identification of distinct basal and luminal subtypes of muscle-invasive bladder cancer with different sensitivities to frontline chemotherapy. Cancer Cell 2014;25(2):152–65.
[11] Fu Y, Liu HW, Forsythe SM, Kogut P, McConville JJ, Halayko AJ, et al. Mutagenesis analysis of human SM22: characterization of actin binding. J Appl Physiol 2000;89 (5):1985–90 1985.
[12] Han M, Dong LH, Zheng B, Shi HJ, Wen JK, Cheng Y. Smooth muscle 22 alpha maintains the differentiated phenotype of vascular smooth muscle cells by inducing filamentous actin bundling. Life Sci 2009;84(13–14):394–401.
[13] Assinder SJ, Stanton JA, Prasad PD. Transgelin: an actin-binding protein and tumour suppressor. Int J Biochem Cell Biol 2009;41(3):482–6.
[21] Chen R, Feng C, Xu Y. Cyclin-dependent kinase-associated protein Cks2 is associated with bladder cancer progression. J Int Med Res 2011;39(2):533–39.

[22] Kawakami K, Enokida H, Tachiwada T, Gotanda T, Tsuneyoshi K, Kubo H, et al. Identification of differentially expressed genes in human bladder cancer through genome-wide gene expression profiling. Oncol Rep 2006;16(3):521–31.

[23] Liu Y, Wu X, Wang J, Hu S, Zhang Y, Zhao S. CALD1, CNNL1, and TAGLN identified as potential prognostic molecular markers of bladder cancer by bioinformatics analysis. Med (Baltimore) 2019;58(2):13847.

[24] Ning X, Deng Y. Identification of key pathways and genes influencing prognosis in bladder urothelial carcinoma. Onco Targets Ther 2017;10:1673–86.

[25] Wright GW, Simon RM. A random variance model for detection of differential gene expression in small microarray experiments. Bioinformatics 2003;19(18):2448–55.

[26] Clarke R, Ressom HW, Wang A, Qiu P. Interaction of Smad3 and SRF-associated complex mediates TGF-β1 signaling to regulate SM22α expression in endothelial cells in response to interleukin-1beta and transforming growth factor-beta. Cell Signal 2015;27(8):1589–96.

[27] Fan Y, Shen B, Tan M, Mu X, Qin Y, Zhang F, et al. TGF-beta-induced upregulation of malat1 promotes bladder cancer metastasis by associating with suz12. Clin Cancer Res 2016;22(1):56.

[28] Aldeiri B, Roostalu M, Dawan DK, RAjaee NM, Hamam R, Alfayez M, et al. Transgelin is a TGFbeta-inducible gene that regulates osteoblastic and adipogenic differentiation of human skeletal stem cells through actin cytoskeleton organisation. Cell Death Dis 2012;19(7):321–37.

[29] Alderri B, Roostalu U, Albertini A, Wong J, Morabito A, Cossu G. Transgelin—expressing myofibroblasts orchestrate ventral midline closure through TGFβ signalling. Development. 2017;144(18):3336–48.

[30] Shariat SF, Kim JH, Andrews B, Kattwinkel W, Wheeler TM, Kim Y, et al. Preoperative plasma levels of transforming growth factor beta1 strongly predict clinical outcome in patients with bladder carcinoma. Cancer 2001;92(12):2985–92.

[31] Pignatelli J, Tumbarello DA, Schmidt RP, Turner CE. Hic-5 promotes invadopodia formation of differentially-expressed proteins between early submucosal non-invasive and invasive colorectal cancer using 2d-Dige and mass spectrometry. Int J Immunopathol Pharmacol 2011;24(4):849–59.

[32] Lawson D, Harrison M, Shapland C. Fibroblast transgelin and smooth muscle SM22 alpha are the same protein, the expression of which is down-regulated in many cell lines. Cell Motil Cytoskeleton 1997;38(3):250–7.

[33] Yang HJ, Liu GL, Liu B, Liu T. GP73 promotes invasion and metastasis of bladder cancer cell lines. Cell Motil Cytoskeleton 2005;59(10):296–304.

[34] Maleszewska M, Gjaltema RA, Krenning G, Harmsen MC. Enhancer of zeste homolog-2 (Ezh2) mitochondrial transgelin regulates transgelin/smooth muscle-2alpha expression in endothelial cells in response to interleukin-Ibeta and transforming growth factor-beta.2 Cell Signal 2015;27(8):1589–96.

[35] Elsafadi M, Manikanand M, Dawud RA, Alajez NM, Hamam R, Alfayez M, et al. Transgelin is a TGFbeta-inducible gene that regulates osteoblastic and adipogenic differentiation of human skeletal stem cells through actin cytoskeleton organization. Cell Death Dis 2016;7(8):e2321.

[36] Ecker MA, Lwin TM, Chang AT, Kim J, Danis E, Ohno-Machado L, et al. Twist1-induced invadopodia formation promotes tumor metastasis. Cancer Cell 2011;19(3):372–86.

[37] Maleszewska M, Gjaltema RA, Krenning G, Harmsen MC. Enhancer of zeste homolog-2 (Ezh2) mitochondrial transgelin regulates transgelin/smooth muscle-2alpha expression in endothelial cells in response to interleukin-Ibeta and transforming growth factor-beta.2 Cell Signal 2015;27(8):1589–96.

[38] Fan Y, Shen B, Tan M, Mu X, Qin Y, Zhang F, et al. TGF-beta-induced upregulation of malat1 promotes bladder cancer metastasis by associating with suz12. Clin Cancer Res 2014;20(6):1531–41.

[39] Yang HJ, Liu GL, Liu B, Liu T. GP73 promotes invasion and metastasis of bladder cancer by regulating the epithelial-mesenchymal transition through the TGFbeta-1 Smad2 signalling pathway. J Cell Mol Med 2018;22(3):1650–65.

[40] Marthasahan S, Turley SJ, Nickles D, Castiglioni A, Yuan K, Wang Y, et al. TGFbeta attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. Nature 2018;554(7693):544–8.

[41] Akhurst RJ, Hata A. Targeting the TGFbeta signalling pathway in disease. Nat Rev Drug Discov 2012;11(10):790–811.

[42] Colak S, Ten Dijke P. Targeting TGF-beta signaling in cancer. Trends Cancer 2017;3(1):56–71.

[43] Qi P. Interaction of Smad3 and SRF-associated complex mediates TGF-β1 signals to regulate SM22α expression during myofibroblast differentiation. J Mol Cell Cardiol 2003;35(12):1407–20.

[44] Chen S. Smad proteins regulate transcriptional induction of the SM22alpha gene by TGF-β. Nucleic Acids Res 2013;41(4):1302–10.

[45] Voutierlon TC, de Jong FC, Costello JC, Theodorescu D. Systematic review: characteristics and preclinical uses of bladder cancer cell lines. Bladder Cancer 2018;4(2):169–83.

[46] Seiler R, Ashab HAD, Erho N, van Rhijn BWG, Winters B, Douglas J, et al. Impact of molecular subtypes in muscle-invasive bladder cancer on predicting response and survival after neoadjuvant chemotherapy. Eur Urol 2017;72(4):544–54.

[47] Cameroni-Mercedes B, Fosythe SM, LeBee MM, Espinosa 3rd, Vieira JF, Halajy AJ, et al. Expression and cytotergenetic localization of the human SM22 gene (TAGLN). Genomics 1998;49(3):452–7.

[48] Lawson D, Morrison H, Shapland C. Fibroblast transgelin and smooth muscle SM22 alpha are the same protein, the expression of which is down-regulated in many cell lines. Cell Motil Cytoskeleton 1997;38(3):250–7.

[49] Zhang J, Song MQ, Zhou JS, Zhou Z, Xu ZP, Chen WX, et al. Identification of differentially-expressed proteins between early submucosal non-invasive and invasive colorectal cancer using 2d-Dige and mass spectrometry. Int J Immunopathol Pharmacol 2011;24(4):849–59.

[50] Kim HJ, Kang UB, Lee H, Jung JH, Lee ST, Yu MH, et al. Profiling of differentially expressed proteins in stage IV colorectal cancers with good and poor outcomes. J Proteomes 2012;75(10):2580–93.