Molecular and Cellular Biochemistry (2020) 465:165–174
https://doi.org/10.1007/s11010-019-03676-8

Transforming growth factor-β1 enhances proliferative and metastatic potential by up-regulating lymphoid enhancer-binding factor 1/integrin αMβ2 in human renal cell carcinoma

Yuting Liu[2] · Donghao Shang[1]

Received: 21 September 2019 / Accepted: 7 December 2019 / Published online: 17 December 2019
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Abstract
Renal cell carcinoma (RCC) is a kind of malignant tumor with high recurrence, and it is urgent to find molecular markers for diagnosis and prognosis of RCC. Our study investigated the expression and function of integrin αMβ2 in RCC cells, aiming to understand the role of integrin αMβ2 in RCC and develop new therapeutic target for RCC. Overexpression and knockdown of lymphoid enhancer-binding factor 1 (LEF1) were performed using vector containing full-length cDNA and via siRNA technology, respectively. The expressions of mRNA and protein were detected by RT-PCR and Western blot, respectively. Proliferation of RCC cell was analyzed using WST-1 assay, and metastasis of RCC cell was evaluated using the transwell system. Our results demonstrated that LEF1 and integrin αMβ2 were up-regulated in RCC cells via TGF-β1-dependent mechanism, and LEF1 together with β-catenin directly increased integrin αMβ2 level. On the other hand, TGF-β1-induced proliferation, migration and invasion were suppressed by function-blocking antibody against integrin αMβ2 in RCC cells. In addition, integrin αMβ2 is crucial for LEF1 mediated cell invasion by regulating MMP-2, MMP-9 and calpain-2 secretion in RCC cells. LEF1/integrin αMβ2 expression was regulated by TGF-β1, and LEF1/integrin αMβ2 was involved in TGF-β1's improvement effects on the proliferation and metastasis of RCC. Blocking integrin αMβ2 activity could be a therapeutic option for patients with advanced RCC.

Keywords Renal · Cell carcinoma · TGF-β1 · LEF1 · Integrin · Proliferation · Metastasis

Introduction

Renal cell carcinoma (RCC) accounts for approximately 2–3% of all malignancies and is the 12th most common cancer worldwide [1–3]. Currently, the only potential curative treatment for localized RCC is surgery; however, 20–30% of patients with RCC experience local or distant recurrence within 5 years after radical nephrectomy [4]. This is a major factor limiting patient survival; therefore, identification of new molecular markers to predict patient survival and tumor relapse remains to be a subject of fundamental importance. At present, studies concerning novel molecular targets in RCC were very limited.

Integrins are heterodimeric transmembrane receptors that could mediate interactions of cells with the extracellular matrix (ECM) [5]. Integrins are formed by specific noncovalent associations between different α and β subunits [6], and each subunit contains a cytoplasmic tail, a transmembrane and an extracellular region [7, 8]. The integrin family is classified according to the associated β-subunit, mainly including β1 (CD29) and β2 (CD18) [9, 10]. The β2 integrin family has a common β2 chain paired with homologous α subunits and consists of the following four members: αMβ2 (CD11b/CD18, or Mac-1); αLβ2 (CD11a/CD18, or LFA-1); αDβ2 (CD11d/CD18); and αXβ2 (CD11c/CD18).

Integrins exhibit a very broad ligand-binding specificity with the component of ECM, which allows for its diverse cell functions, such as cell interactions, adhesion and migration [11, 12]. Abnormal expression of integrins often correlates with irregular processes like inflammation or tumor. Integrin αMβ2 is mainly expressed in myeloid, NK and T

✉ Donghao Shang
kyotosdh@163.com

[1] Department of Urology, Friendship Hospital, Capital Medical University, Beijing 100050, China

[2] Department of Pathology, Capital Medical University, Beijing 100069, China

cells [6], which not only participate in regulating monocyte differentiation and mediating adhesive reactions of leukocytes during the inflammatory response [7, 13, 14], but also take part in the maintenance of tolerance and control of inflammation [15, 16]. However, the specific roles of integrin αMβ2 in the progression of tumor cells remain unclear.

Our previous study inspired us that LEF1 and the integrin αMβ2 may be related to RCC cells, which still needs to be confirmed in further study [17, 18]. LEF1, initially identified as a pre-B and T lymphoid-specific gene belonging to the family of high-mobility group transcription factors [19, 20], contains a strong DNA binding domain near the C terminus and a domain at the N terminus that binds the transcription activator, β-catenin [21]. Chang et al. indicated that β-catenin could regulate integrin α5β1 expression, and LEF1 binding sites in the promoter regions of integrin α5/β1 were also confirmed [22]. Thus, whether LEF1 is involved in the regulation of integrin αMβ2 in RCC should be further investigated.

The TGF-β signaling pathway has been confirmed to modulate numerous physiologic processes, including proliferation, migration and invasion of tumors [23–25], and TGF-β1 is involved in promoting the proliferation of RCC cells [26–28]. Moreover, Lebdai et al. identified and validated TGF-β1 as a promising prognosis marker of clear cell renal cell carcinoma [29], and Huang et al. found TGF-β1 could induce Fascin1 to promote cell invasion and metastasis of human 786-0 RCC cells [30]. Previous study demonstrated that Smad7 interacted with β-catenin and LEF1/TCF, transcriptional regulators in Wnt signaling, in a TGF-β-dependent manner [31]. Also, integrin signaling was found to potentiate TGF-β1 with important implications for epithelial to mesenchymal transition (EMT) in RCC [32]. However, it is still unknown whether TGF-β1 participated in regulating LEF1/integrin αMβ2 expression in RCC cells. In this study, we tried to figure out whether integrin αMβ2 is associated with RCC and its detail role in the development of RCC. We assume that TGF-β1 may employ LEF1/integrin αMβ2 to further enhance the proliferative and metastatic potential in human renal cell.

Material and method

Cell culture and agents

Four commercially available human RCC cell lines (ACHN, Caki-1, NC 65 and A498) were obtained from the ATCC (Manassas, VA, USA). All cells were incubated with complete medium, consisting of 10% heat-inactivated fetal bovine serum and supplemented with RPMI-1640, 2 mM L-glutamine, 1% nonessential amino acids, 25 mM HEPES and penicillin (100 U/ml)/streptomycin (100 μg/ml) (Sigma-Aldrich, St. Louis, MO, USA). RCC cell lines were cultured as a monolayer in an incubator at 37 °C with a humidified atmosphere of 5% CO_2. TGF-β1 was purchased from Sigma-Aldrich, St Louis, MO, USA, and RCC cells were treated with TGF-β1 (10 ng/ml) in the following experiments.

Reverse transcription-PCR

Total RNA of RCC cells was isolated using Trizol reagent (Invitrogen, Carlsbad, CA, USA). First-strand cDNA synthesis was performed using a cDNA synthesis kit (Applied Biosystems, Carlsbad, CA, USA). The cDNA was amplified by PCR using TaqMan gene expression assays, and the PCR conditions were set according to the manufacturer's instructions (Applied Biosystems). The PCR product was separated by 3% agarose gel electrophoresis, and GAPDH was used as the internal control. All primer sequences in this study are shown in Table 1.

Western blot and immunoprecipitation

Total protein was extracted with protease inhibitor (Roche, Basel, Switzerland) and cell lysis buffer (Cell Signaling, Cambridge, UK). Protein (60 mg/well) was separated by SDS–polyacrylamide gel electrophoresis; then, proteins were transferred to 0.2 μm nitrocellulose (Life Technologies, Carlsbad, CA, USA) and incubated with a blocking solution for 2 h at room temperature. The membranes were incubated with primary antibodies overnight at 4 °C. Integrin αM/CD11B (D6X1N) rabbit mAb, integrin β2 (D4N5Z) rabbit mAb, LEF1 (C12A5) rabbit mAb, β-catenin (D10A8) XP® rabbit mAb, mouse (G3A1) mAb IgG isotype control, matrix metalloproteinases (MMPs)-2 (D8N9Y) rabbit mAb and MMP-9 (D6O3H) XP® rabbit mAb were purchased from Cell Signaling Technology. β-actin monoclonal antibody (ab6276) and calpain 2 antibody (ab39165) were purchased from Abcam (Cambridge, UK). The immune complexes were detected with an ECL system (Amersham, Aylesbury, UK) according to the manufacturer's instructions. Integrin function-blocking monoclonal antibodies (CD18 (CBL158) and CD11b (CBL145)) were obtained from Chemicon (Temecula, CA, USA).

RNA interference and transfection

The siRNA oligonucleotide or scrambled siRNA (negative control) was designed using siDirect software. The oligonucleotides used in this study are shown in Table 1. RCC cells were seeded at the density of 1×10^5 cells per well into a 6-well culture dish and incubated until confluence reached 50–60%; then, cells were transfected with siRNA oligonucleotides by Lipofectamine 2000 reagent (Invitrogen,

Table 1 Primer sequences and siRNA oligonucleotides used in this study

Primers	Forward primer (5′–3′)	Reverse primer (5′–3′)	Length of PCR products (bp)
Integrin αM	CTGAACGTCACTCTTGTGCA	CCTCTTGAGGACACCCTCGG	110
Integrin β2	GGTAGTAGACGAGTCCCGAC	GCTCACAGTTGATGGTGTCA	120
LEF1	GGTCGGACTGAGTGTGTGTG	AGTTTTTGCCGGCAAGCGCG	132
TGF-β1	TCTGGGAAAGAGGAGGT	CTCAGTATCCCACGGAAATA	120
GAPDH	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTC	226
siRNA	Sense oligonucleotide (5′–3′)	Antisense oligonucleotide (5′–3′)	Target gene sequence (5′–3′)
LEF1	AAGAGAAAGAGAAGUUUGCC	GCAAACUUCUCUUUCUCUUCC	TGGCAAACT TCTTTC TCTTCT
Negative control	GUACCGCACGUCAUUCGUAUC	UACGAAUGACGUGCGGUACGU	

Carlsbad, CA, USA). The cDNA coding sequence of LEF1 was cloned, as previously described [33]. RCC cell lines were also transfected with LEF1 vector containing full-length cDNA for LEF1 or with a blank vector without inserting the LEF1 by Lipofectamine 2000. RCC monoclonal cell lines were selected by G418, and the expression of LEF1 was detected using Western blot analysis.

Cell viability assay

The proliferative ability of RCC cells was analyzed using WST-1 assay. Briefly, RCC cells were seeded into 96-well plates at a density of 0.5×10^4 cells per well. After 48 h of continuous incubation, 20 µL of WST-1 reagent (Roche, Penzberg, Germany) was added to each well. Following incubation for 2 h at 37 °C, the viable cells were detected by measuring absorbance at 450 nm using an absorbance reader (Immunoreader NJ-2000; Japan Intermed, Tokyo, Japan).

Cell migration and invasion assays

For the migration assays, chemotaxis was detected using a Transwell system (Poretics Corp., Livermore, CA, USA) containing 8-µm pore polycarbonate membrane filters. The invasion assay was analyzed using a Transwell system incorporating a polycarbonate filter membrane (Corning, NY, USA). Briefly, 1×10^5 RCC cells were selected in 100 µL of serum-free medium and seeded into the upper chamber. After continuous incubation at 37 °C for 48 h, the invading cells on the bottom of each well and the migrating cells in the lower chamber were fixed with methyl alcohol, and the number of RCC cells was counted by a CX23 microscope (Olympus Corporation, Tokyo, Japan) in five randomly microscopic fields.

Statistical analysis

Statistical analysis was performed using SPSS 16.0 (SPSS, Inc., Chicago, IL, USA). All results in this study were shown as the mean ± standard deviation (SD). Comparisons between two groups were made by unpaired or paired Student's *t* tests. All statistical tests were 2-tailed, and p value < 0.05 was regarded as significant different.

Results

RCC cells possess higher expression of LEF1 and integrin αMβ via TGF-β1-dependent mechanism

We employed RT-PCR and Western blot to detect the expression of LEF1, integrin αMβ2 and TGF-β1 in four pairs of RCC and corresponding normal kidney tissue. Our results demonstrated that the expression of LEF1, integrin αMβ2 and TGF-β1 was up-regulated in RCC compared to normal kidney tissue (Fig. 1), which suggested their involvement in RCC development. Of the four RCC cell lines, the result was consistent, and ACHN and Caki-1 were selected for following experiments (Fig. 2a, b). To determine whether TGF-β1 exerts its affection on LEF1 and integrin αMβ2, TGF-β1 (10 ng/ml for 48 h) was added to ACHN and Caki-1 for further detection of protein expression. The results showed that TGF-β1 could significantly up-regulate the expression of LEF1 and integrin αMβ2 in RCC cells (Fig. 2c). These findings suggested that TGF-β1-dependent mechanism in RCC cells may contribute greatly to the up-regulation of LEF1 and integrin αMβ2.

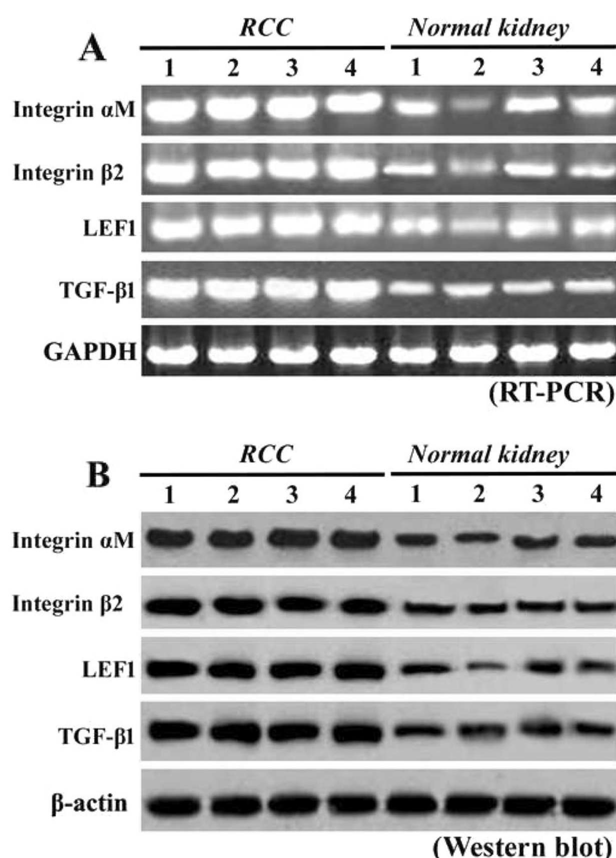

Fig. 1 The expression of LEF1, integrin αMβ2 and TGF-β1 was detected by RT-PCR (**a**) and Western blot (**b**) in RCC and normal kidney with four pairs of samples shown

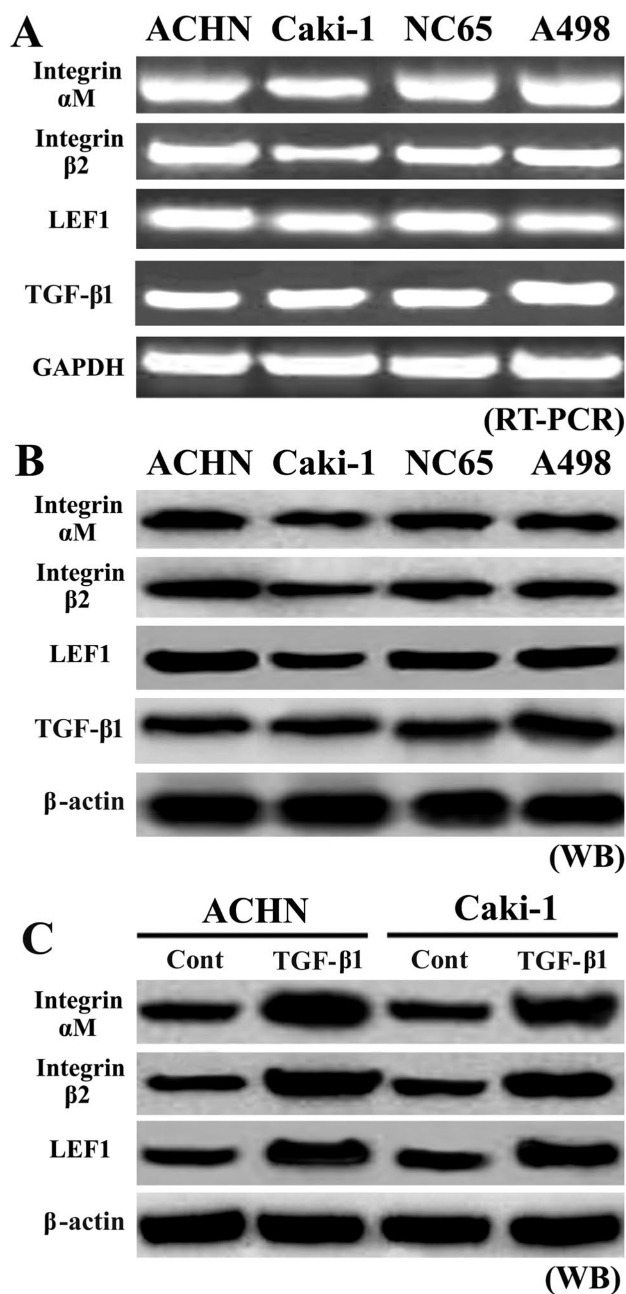

Fig. 2 The expression of LEF1 and integrin αMβ2 was detected by RT-PCR (**a**) and Western blot (**b**), and TGF-β1 increased the expression of LEF1 and integrin αMβ2 in RCC cells (C)

LEF1 may interact with β-catenin and regulate the expression of integrin αMβ2

We investigated the relationship among LEF1, integrin αMβ2 and TGF-β1 to see how the three proteins affect each other. Firstly, overexpression and knockdown of LEF1 in RCC cells were achieved by different vectors, and Western blot was used to detect the change of three protein expression. As demonstrated, the protein level of integrin αMβ2 was significantly increased in RCC cells with high expression of LEF1, whereas markedly decreased in LEF1 knockdown RCC cells. Meanwhile, the expression of TGF-β1 was not affected by LEF1 transfections in RCC cells. In addition, RCC cell lines with varying expression of LEF1 were subjected to immunoprecipitation test to evaluate the interaction of LEF1 and β-catenin. Although LEF1 did not affect β-catenin expression, higher amount of LEF1/β-catenin complex was detected in RCC cells with high expression of LEF1 (Fig. 3). These findings suggested that LEF1 may interact with β-catenin and regulate the expression of integrin αMβ2 in human RCC cells.

LEF1 enhances the proliferation and metastasis of RCC cells

The proliferation and metastasis are considered as important steps in the development of RCC, so the proliferative and metastatic potential of RCC cells were evaluated concerning the LEF1 status. As shown in Fig. 4a, the effect of LEF1 on the proliferation of RCC cells was analyzed by the WST-1 assay. RCC cell lines with overexpressed LEF1 showed

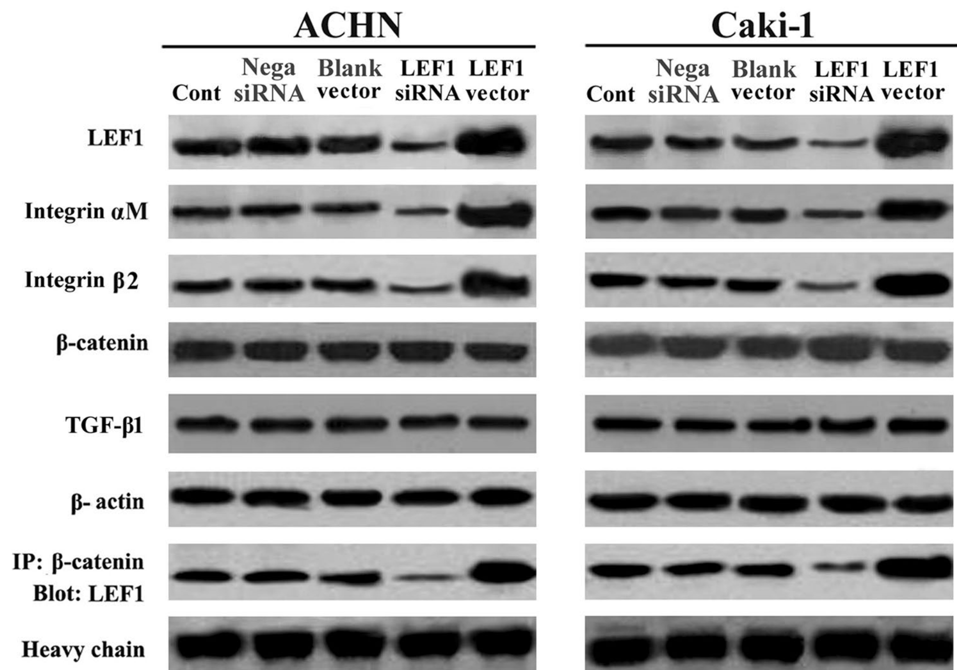

Fig. 3 LEF1 regulated the expression of integrin αMβ2 in RCC cells, and the expression of LEF1 was detected by Western blot and immunoprecipitation after transfection with LEF1 siRNA oligonucleotide and a vector containing full-length cDNA for LEF1

increased proliferative ability compared to the control cells, but significantly reduced proliferative ability upon knockdown of LEF1. In addition, RCC cells with lower expression of LEF1 had less capacity for migration and invasion than those cell lines with higher expression of LEF1 (Fig. 4b, c). These results suggested that LEF1 was involved in the proliferation and metastasis of RCC cells.

Integrin αMβ2 is necessary for TGF-β1-induced proliferation and metastasis of human RCC

TGF-β1 plays an important role in the carcinogenesis of RCC; however, whether its function depends on integrin αMβ2 is unclear, so blocking antibody against integrin αM or β2 was used to detect the effect that integrin αMβ2 exerts on TGF-β1 function. As shown in Fig. 5a–c, RCC cells treated with TGF-β1 (10 ng/ml) for 48 h significantly improved cell growth and enhanced cell migration and invasion capacity. But administration of blocking antibodies significantly decreased proliferative ability of untreated cells and TGF-β1 treated cells (Fig. 5a), and similar results were found in regard as cell migration and invasion capacity.

These findings indicated that integrin αMβ2 was necessary for TGF-β1-induced proliferation and metastasis of human RCC.

Integrin αMβ2 is crucial for LEF1 up-regulating the expression of MMPs and calpain-2

The effect of LEF1 and integrin αMβ2 on regulation of MMP2, MMP9 and calpain-2 expression was evaluated in this study, which aimed to further investigate the molecular mechanism of LEF1/integrin αMβ2 involved in the metastasis of RCC cells. Our results found that the amount of LEF1 was positively associated with the expression of MMPs and calpain-2, mainly embodied by overexpression of LEF1 with increased MMPs and calpain-2 expression, and knockdown of LEF1 with decreased MMPs and calpain-2 expression (Fig. 6a). However, MMPs and calpain-2 expression could be suppressed after treatment with blocking antibody against integrin αM or β2 (1 μg/ml) for 48 h in LEF1 highly expressed RCC cells (Fig. 6b). These findings suggested that LEF1 enhanced the metastatic potential depending on the regulation of MMPs and calpain-2 secretion by integrin αMβ2 in human RCC.

Discussion

Our study gained a new finding that integrin αMβ2 positively promoted RCC development, based on its up-regulation in RCC cells and facilitating proliferative and metastatic potential of RCC cells. This helped to amplify function of integrin αMβ2 besides its regulation on inflammation and shed light on understanding and control of RCC.

The positive correlation between integrin αMβ2 expression and LEF1 suggested that LEF1 may directly act as the transcription factor for αMβ2 to govern αMβ2 expression, and this should be more definite if the evidence that LEF1 has binding sites in the promoter regions of integrin αMβ2 is added. As known, LEF1 asks for other transcription activators to exert its function, and LEF1/β-catenin complex

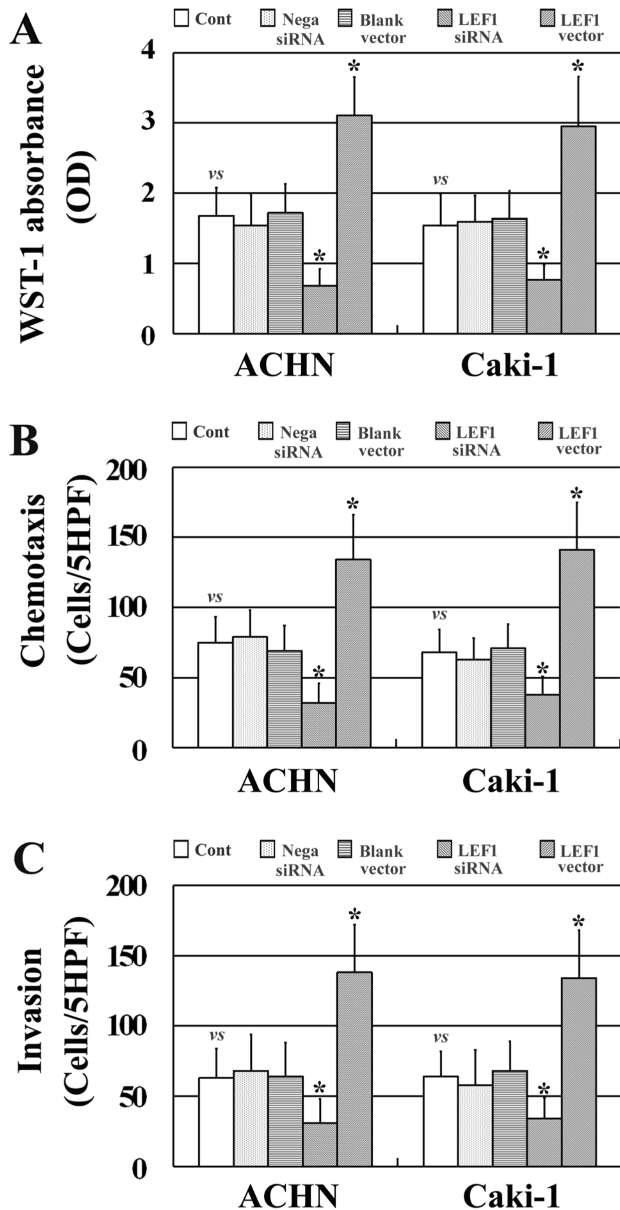

Fig. 4 LEF1 played an important role in the proliferation (**a**), migration (**b**) and invasion (**c**) of RCC cells. All experiments were performed in triplicate, and the error bar represents the SD. *$p < 0.05$ vs control

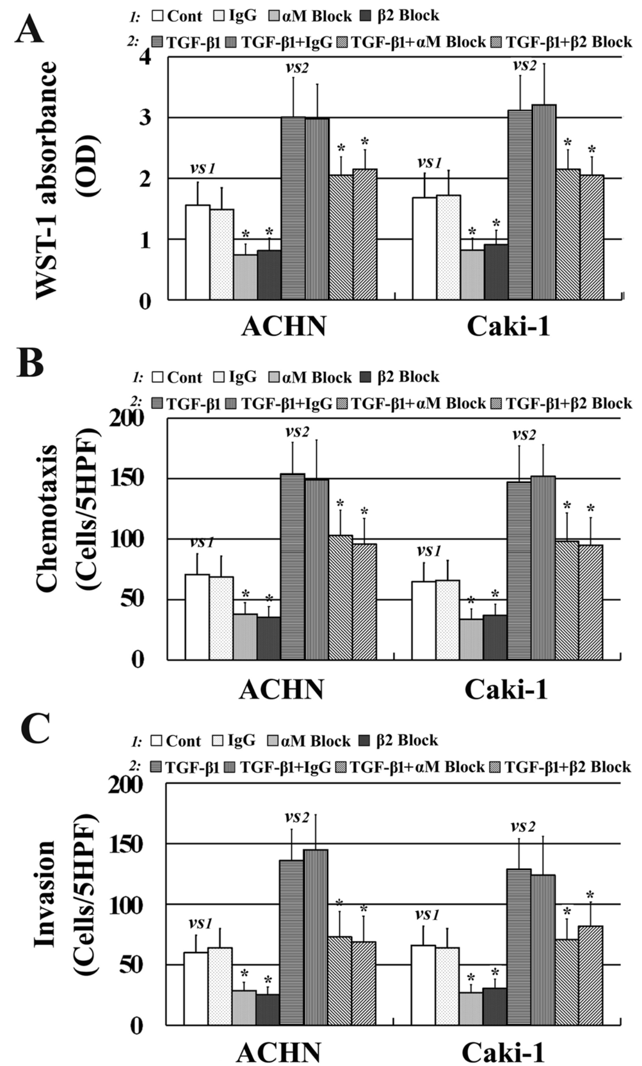

Fig. 5 TGF-β1 induced the proliferation (**a**), migration (**b**) and invasion (**c**) depended on the activity of integrin αMβ2 in RCC. All experiments were performed in triplicate, and the error bar represents the SD. *$p < 0.05$ vs 1. Control vs 2. TGF-β1

is confirmed to act downstream of the Wnt/β-catenin signaling [34, 35], which is regarded as great contribution for tumor cells progression [36]. Here in our study, accordingly, LEF1 formed complex with β-catenin to up-regulate integrin αMβ2 in RCC cells. Consequently, as the key component of Wnt/β-catenin pathway, LEF1 could enhance the proliferation, migration and invasion of RCC cells, largely owning to its up-regulation of genes related to these processes, of course, including integrin αMβ2.

Interestingly, we also found that TGF-β1 stimulation could up-regulate the expression of LEF1 and integrin αMβ2 in RCC cells, which may point out the cross talk between TGF-β1 and Wnt/β-catenin pathway just as reported [37]. As known, TGF-β mainly exerts its functions through classical SMAD-dependent mechanism. Reports showed that SMAD3 jointed the complex of LEF1/β-catenin upon TGF-β1 stimulation, and triggered up-regulation of β1 integrin gene expression [33, 38]. Similarly, the complex of LEF1/β-catenin/SMAD7 leads to the up-regulation of LEF1 itself [39]. Here, in RCC cells, it could be deduced that TGF-β1 signaling promotes Wnt/β-catenin pathway, leading to up-regulation of transcription factor LEF1, which served as a positive feedback, and enlarges targeted genes related to tumor progression, including LEF1 itself and integrin αMβ2.

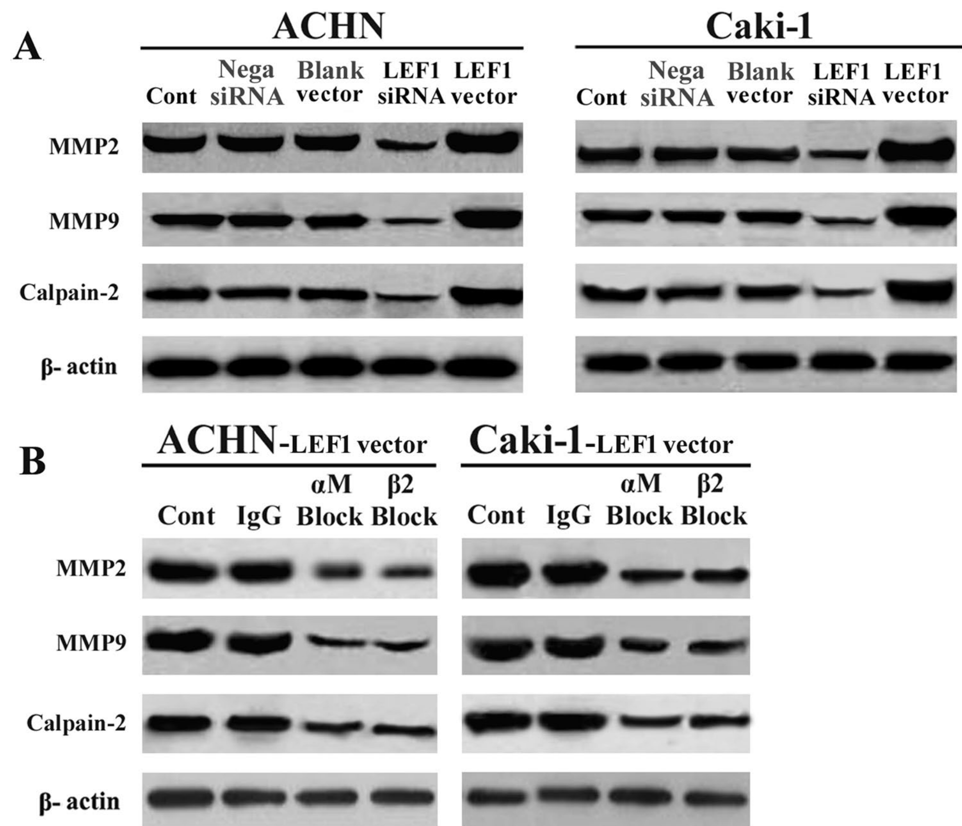

Fig. 6 LEF1 enhanced the metastatic potential by up-regulating MMP-2, MMP-9 and calpain-2 secretion depending on the activity of integrin αMβ2 in RCC

Integrin αMβ2, as a transmembrane receptor itself, has cross talk with TGF-β1 signal. To be noted, integrins could extracellularly activate TGF-β1 which is secreted in a latent form failing to trigger receptor mediated TGF-β signaling [40]. I interacts with TGF-β receptor (TβR) type II, and the interaction enhances TGF-β stimulation of MAPKs and Smad2/3-mediated gene transcription, thereby significantly promoting TGF-β induced EMT in tumor cell [41]. Additionally, cross talk between TGF-β and integrin signaling can also occur on downstream receptors, mainly through affecting the common signal molecules related to the two pathways [42, 43]. All the above information may inspire that TGF-β signaling up-regulates the expression of integrin αMβ2, and then αMβ2 served as a positive feedback, aiming to facilitate and enhance TGF-β stimulated signaling. Finally, the fact in our study that blocking antibody against integrin αMβ2 suppressed TGF-β1's effects on RCC cells, may be clearly due to the blocker attenuated αMβ2 facilitating TGF-β1 induced EMT, a key index featuring migration and invasion of tumor cells.

Apart from assisting TGF-β signal, integrins govern pathways mediated by its own to transduce the extracellular survival and invasion signal [43]. Though lack of kinase activity, when activated by ligand in ECM, integrin could recruit diverse kinases, including focal adhesion kinase, integrin linked kinase and the SRC kinase family, to induce the cascade signal transduction involving Raf-ERK/MAPK and PI3K/AKT pathway. MMP2 and MMP9 are included in gelatinases belonging to MMPs. Previous studies indicated that MMP2 could mediate migration of vascular smooth muscle cell [44] and enhance pericellular proteolysis and invasion [45]. Downregulation of MMP2 and MMP9 was involved in the inhibition of migration and invasion in RCC cells [46]. Also, calpain-2 has been reported to mediate the invasion of glioma cells and possibly regulate MMP2 [47]. MMPs could just be the targeted prey of integrins through the mentioned pathways [48–50], who belong to proteinase family with biological functions in tumor migration and invasion [45, 51–53]. This is in accordance with our finding that expression of MMP2 and MMP9 is quite dependent on the status of integrin αMβ2 despite of the overexpression of LEF1. Taken the fact that TGF-β meditating classical SMAD and nonclassical pathways largely contributes to the EMT related gene expression including MMPs [54], it could be deduced that TGF-β together with integrins should be the determinant factors toward EMT and invasion of tumor cells.

In conclusion, our study suggested that integrin αMβ2 up-regulation in RCC cells was dependent on combined effect of TGF-β1 and Wnt/β-catenin pathway leading to high amount of LEF1. Also, we found that integrin αMβ2 played an essential and crucial role in the proliferation, migration and invasion of RCC cells, mainly through assisting TGF-β1

stimulated signal and by its own. All supported the conclusion that TGF-β1 strengthens proliferative and metastatic potential by means of up-regulating LEF1/integrin αMβ2 in human renal cell. These results also suggested that blocking integrin αMβ2 activity could be a new therapeutic option for patients with advanced RCC. Of course, the molecular mechanisms and the expression of integrin αMβ2 in RCC need further investigation.

Funding This study was supported by National Science Foundation of China (No. 81572502).

Compliance with ethical standards

Conflict of interest All the authors declare that they have no conflict of interest.

References

1. Porta C, Paglino C, Grunwald V (2014) Sunitinib re-challenge in advanced renal-cell carcinoma. Br J Cancer 111(6):1047–1053. https://doi.org/10.1038/bjc.2014.214
2. Chiong E, Tay MH, Tan MH, Kumar S, Sim HG, Teh BT, Umbas R, Chau NM (2012) Management of kidney cancer in Asia: resource-stratified guidelines from the Asian Oncology Summit 2012. Lancet Oncol 13(11):e482–491. https://doi.org/10.1016/S1470-2045(12)70433-3
3. Patard JJ, Leray E, Rioux-Leclercq N, Cindolo L, Ficarra V, Zisman A, De La Taille A, Tostain J, Artibani W, Abbou CC, Lobel B, Guille F, Chopin DK, Mulders PF, Wood CG, Swanson DA, Figlin RA, Belldegrun AS, Pantuck AJ (2005) Prognostic value of histologic subtypes in renal cell carcinoma: a multicenter experience. J Clin Oncol 23(12):2763–2771. https://doi.org/10.1200/JCO.2005.07.055
4. Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Oudard S, Negrier S, Szczylik C, Pili R, Bjarnason GA, Garcia-del-Muro X, Sosman JA, Solska E, Wilding G, Thompson JA, Kim ST, Chen I, Huang X, Figlin RA (2009) Overall survival and updated results for sunitinib compared with interferon alfa in patients with metastatic renal cell carcinoma. J Clin Oncol 27(22):3584–3590. https://doi.org/10.1200/JCO.2008.20.1293
5. Wu D, Xu Y, Ding T, Zu Y, Yang C, Yu L (2017) Pairing of integrins with ECM proteins determines migrasome formation. Cell Res 27(11):1397–1400. https://doi.org/10.1038/cr.2017.108
6. Barczyk M, Carracedo S, Gullberg D (2010) Integrins. Cell Tissue Res 339(1):269–280. https://doi.org/10.1007/s00441-009-0834-6
7. Rucci N, Teti A (2016) The "love-hate" relationship between osteoclasts and bone matrix. Matrix Biol 52–54:176–190. https://doi.org/10.1016/j.matbio.2016.02.009
8. Hynes RO (2002) Integrins: bidirectional, allosteric signaling machines. Cell 110(6):673–687. https://doi.org/10.1016/s0092-8674(02)00971-6
9. Streuli CH (2009) Integrins and cell-fate determination. J Cell Sci 122(Pt 2):171–177. https://doi.org/10.1242/jcs.018945
10. Abram CL, Lowell CA (2009) The ins and outs of leukocyte integrin signaling. Annu Rev Immunol 27:339–362. https://doi.org/10.1146/annurev.immunol.021908.132554
11. Prieto J, Eklund A, Patarroyo M (1994) Regulated expression of integrins and other adhesion molecules during differentiation of monocytes into macrophages. Cell Immunol 156(1):191–211. https://doi.org/10.1006/cimm.1994.1164
12. Shang XZ, Issekutz AC (1997) Beta 2 (CD18) and beta 1 (CD29) integrin mechanisms in migration of human polymorphonuclear leucocytes and monocytes through lung fibroblast barriers: shared and distinct mechanisms. Immunology 92(4):527–535. https://doi.org/10.1046/j.1365-2567.1997.00372.x
13. Graff JC, Jutila MA (2007) Differential regulation of CD11b on gammadelta T cells and monocytes in response to unripe apple polyphenols. J Leukoc Biol 82(3):603–607. https://doi.org/10.1189/jlb.0207125
14. Shi C, Sakuma M, Mooroka T, Liscoe A, Gao H, Croce KJ, Sharma A, Kaplan D, Greaves DR, Wang Y, Simon DI (2008) Down-regulation of the forkhead transcription factor Foxp1 is required for monocyte differentiation and macrophage function. Blood 112(12):4699–4711. https://doi.org/10.1182/blood-2008-01-137018
15. Shi C, Zhang X, Chen Z, Sulaiman K, Feinberg MW, Ballantyne CM, Jain MK, Simon DI (2004) Integrin engagement regulates monocyte differentiation through the forkhead transcription factor Foxp1. J Clin Investig 114(3):408–418. https://doi.org/10.1172/JCI21100
16. Xue ZH, Zhao CQ, Chua GL, Tan SW, Tang XY, Wong SC, Tan SM (2010) Integrin alphaMbeta2 clustering triggers phosphorylation and activation of protein kinase C delta that regulates transcription factor Foxp1 expression in monocytes. J Immunol 184(7):3697–3709. https://doi.org/10.4049/jimmunol.0903316
17. Varga G, Balkow S, Wild MK, Stadtbaeumer A, Krummen M, Rothoeft T, Higuchi T, Beissert S, Wethmar K, Scharffetter-Kochanek K, Vestweber D, Grabbe S (2007) Active MAC-1 (CD11b/CD18) on DCs inhibits full T-cell activation. Blood 109(2):661–669. https://doi.org/10.1182/blood-2005-12-023044
18. Skoberne M, Somersan S, Almodovar W, Truong T, Petrova K, Henson PM, Bhardwaj N (2006) The apoptotic-cell receptor CR3, but not alphavbeta5, is a regulator of human dendritic-cell immunostimulatory function. Blood 108(3):947–955. https://doi.org/10.1182/blood-2005-12-4812
19. Santoso S, Sachs UJ, Kroll H, Linder M, Ruf A, Preissner KT, Chavakis T (2002) The junctional adhesion molecule 3 (JAM-3) on human platelets is a counterreceptor for the leukocyte integrin Mac-1. J Exp Med 196(5):679–691. https://doi.org/10.1084/jem.20020267
20. Simon DI, Chen Z, Xu H, Li CQ, Dong J, McIntire LV, Ballantyne CM, Zhang L, Furman MI, Berndt MC, Lopez JA (2000) Platelet glycoprotein ibalpha is a counterreceptor for the leukocyte integrin Mac-1 (CD11b/CD18). J Exp Med 192(2):193–204. https://doi.org/10.1084/jem.192.2.193
21. Waterman ML, Fischer WH, Jones KA (1991) A thymus-specific member of the HMG protein family regulates the human T cell receptor C alpha enhancer. Genes Dev 5(4):656–669. https://doi.org/10.1101/gad.5.4.656
22. Travis A, Amsterdam A, Belanger C, Grosschedl R (1991) LEF-1, a gene encoding a lymphoid-specific protein with an HMG domain, regulates T-cell receptor alpha enhancer function [corrected]. Genes Dev 5(5):880–894. https://doi.org/10.1101/gad.5.5.880
23. Liu LJ, Yu JJ, Xu XL (2017) MicroRNA-93 inhibits apoptosis and promotes proliferation, invasion and migration of renal cell carcinoma ACHN cells via the TGF-beta/Smad signaling pathway by targeting RUNX3. Am J Transl Res 9(7):3499–3513
24. Sjolund J, Bostrom AK, Lindgren D, Manna S, Moustakas A, Ljungberg B, Johansson M, Fredlund E, Axelson H (2011) The notch and TGF-beta signaling pathways contribute to the aggressiveness of clear cell renal cell carcinoma. PLoS ONE 6(8):e23057. https://doi.org/10.1371/journal.pone.0023057

25. Bostrom AK, Lindgren D, Johansson ME, Axelson H (2013) Effects of TGF-beta signaling in clear cell renal cell carcinoma cells. Biochem Biophys Res Commun 435(1):126–133. https://doi.org/10.1016/j.bbrc.2013.04.054
26. Huang F, Newman E, Theodorescu D, Kerbel RS, Friedman E (1995) Transforming growth factor beta 1 (TGF beta 1) is an autocrine positive regulator of colon carcinoma U9 cells in vivo as shown by transfection of a TGF beta 1 antisense expression plasmid. Cell Growth Differ 6(12):1635–1642
27. Wang J, Chen J, Zhang K, Zhao Y, Nor JE, Wu J (2011) TGF-beta1 regulates the invasive and metastatic potential of mucoepidermoid carcinoma cells. J Oral Pathol Med 40(10):762–768. https://doi.org/10.1111/j.1600-0714.2011.01051.x
28. Ananth S, Knebelmann B, Gruning W, Dhanabal M, Walz G, Stillman IE, Sukhatme VP (1999) Transforming growth factor beta1 is a target for the von Hippel-Lindau tumor suppressor and a critical growth factor for clear cell renal carcinoma. Cancer Res 59(9):2210–2216
29. Lebdai S, Verhoest G, Parikh H, Jacquet SF, Bensalah K, Chautard D, Rioux-Leclercq N, Azzouzi AR, Bigot P (2015) Identification and validation of TGFBI as a promising prognosis marker of clear cell renal cell carcinoma. Urol Oncol 33(2):69.e11–69.e68. https://doi.org/10.1016/j.urolonc.2014.06.005
30. Huang W, Cen S, Kang XL, Wang WF, Wang Y, Chen X (2016) TGF-beta1-induced Fascin1 promotes cell invasion and metastasis of human 786-0 renal carcinoma cells. Acta Histochem 118(2):144–151. https://doi.org/10.1016/j.acthis.2015.12.005
31. Edlund S, Lee SY, Grimsby S, Zhang S, Aspenstrom P, Heldin CH, Landstrom M (2005) Interaction between Smad7 and beta-catenin: importance for transforming growth factor beta-induced apoptosis. Mol Cell Biol 25(4):1475–1488. https://doi.org/10.1128/MCB.25.4.1475-1488.2005
32. Feldkoren B, Hutchinson R, Rapoport Y, Mahajan A, Margulis V (2017) Integrin signaling potentiates transforming growth factor-beta 1 (TGF-beta1) dependent down-regulation of E-Cadherin expression—important implications for epithelial to mesenchymal transition (EMT) in renal cell carcinoma. Exp Cell Res 355(2):57–66. https://doi.org/10.1016/j.yexcr.2017.03.051
33. Yeh YC, Wei WC, Wang YK, Lin SC, Sung JM, Tang MJ (2010) Transforming growth factor-{beta}1 induces Smad3-dependent {beta}1 integrin gene expression in epithelial-to-mesenchymal transition during chronic tubulointerstitial fibrosis. Am J Pathol 177(4):1743–1754. https://doi.org/10.2353/ajpath.2010.091183
34. Li TW, Ting JH, Yokoyama NN, Bernstein A, van de Wetering M, Waterman ML (2006) Wnt activation and alternative promoter repression of LEF1 in colon cancer. Mol Cell Biol 26(14):5284–5299. https://doi.org/10.1128/MCB.00105-06
35. Santiago L, Daniels G, Wang D, Deng FM, Lee P (2017) Wnt signaling pathway protein LEF1 in cancer, as a biomarker for prognosis and a target for treatment. Am J Cancer Res 7(6):1389–1406
36. Vallee A, Lecarpentier Y, Vallee JN (2019) Targeting the canonical WNT/beta-catenin pathway in cancer treatment using non-steroidal anti-inflammatory drugs. Cells. https://doi.org/10.3390/cells8070726
37. Guo X, Wang XF (2009) Signaling cross-talk between TGF-beta/BMP and other pathways. Cell Res 19(1):71–88. https://doi.org/10.1038/cr.2008.302
38. Amini Nik S, Ebrahim RP, Van Dam K, Cassiman JJ, Tejpar S (2007) TGF-beta modulates beta-Catenin stability and signaling in mesenchymal proliferations. Exp Cell Res 313(13):2887–2895. https://doi.org/10.1016/j.yexcr.2007.05.024
39. Liu W, Rui H, Wang J, Lin S, He Y, Chen M, Li Q, Ye Z, Zhang S, Chan SC, Chen YG, Han J, Lin SC (2006) Axin is a scaffold protein in TGF-beta signaling that promotes degradation of Smad7 by Arkadia. EMBO J 25(8):1646–1658. https://doi.org/10.1038/sj.emboj.7601057
40. Munger JS, Huang X, Kawakatsu H, Griffiths MJ, Dalton SL, Wu J, Pittet JF, Kaminski N, Garat C, Matthay MA, Rifkin DB, Sheppard D (1999) The integrin alpha v beta 6 binds and activates latent TGF beta 1: a mechanism for regulating pulmonary inflammation and fibrosis. Cell 96(3):319–328. https://doi.org/10.1016/s0092-8674(00)80545-0
41. Galliher AJ, Schiemann WP (2006) Beta3 integrin and Src facilitate transforming growth factor-beta mediated induction of epithelial-mesenchymal transition in mammary epithelial cells. Breast Cancer Res 8(4):R42. https://doi.org/10.1186/bcr1524
42. Mamuya FA, Duncan MK (2012) aV integrins and TGF-beta-induced EMT: a circle of regulation. J Cell Mol Med 16(3):445–455. https://doi.org/10.1111/j.1582-4934.2011.01419.x
43. Desgrosellier JS, Cheresh DA (2010) Integrins in cancer: biological implications and therapeutic opportunities. Nat Rev Cancer 10(1):9–22. https://doi.org/10.1038/nrc2748
44. Belo VA, Guimaraes DA, Castro MM (2015) Matrix metalloproteinase 2 as a potential mediator of vascular smooth muscle cell migration and chronic vascular remodeling in hypertension. J Vasc Res 52(4):221–231. https://doi.org/10.1159/000441621
45. Sato A, Nagase H, Obinata D, Fujiwara K, Fukuda N, Soma M, Yamaguchi K, Kawata N, Takahashi S (2013) Inhibition of MMP-9 using a pyrrole-imidazole polyamide reduces cell invasion in renal cell carcinoma. Int J Oncol 43(5):1441–1446. https://doi.org/10.3892/ijo.2013.2073
46. Chen SJ, Yao XD, Peng BO, Xu YF, Wang GC, Huang J, Liu M, Zheng JH (2016) Epigallocatechin-3-gallate inhibits migration and invasion of human renal carcinoma cells by downregulating matrix metalloproteinase-2 and matrix metalloproteinase-9. Exp Ther Med 11(4):1243–1248. https://doi.org/10.3892/etm.2016.3050
47. Jang HS, Lal S, Greenwood JA (2010) Calpain 2 is required for glioblastoma cell invasion: regulation of matrix metalloproteinase 2. Neurochem Res 35(11):1796–1804. https://doi.org/10.1007/s11064-010-0246-8
48. Li X, Yang Y, Hu Y, Dang D, Regezi J, Schmidt BL, Atakilit A, Chen B, Ellis D, Ramos DM (2003) Alphavbeta6-Fyn signaling promotes oral cancer progression. J Biol Chem 278(43):41646–41653. https://doi.org/10.1074/jbc.M306274200
49. Niu J, Gu X, Turton J, Meldrum C, Howard EW, Agrez M (1998) Integrin-mediated signalling of gelatinase B secretion in colon cancer cells. Biochem Biophys Res Commun 249(1):287–291. https://doi.org/10.1006/bbrc.1998.9128
50. Li Y, Ren Z, Wang Y, Dang YZ, Meng BX, Wang GD, Zhang J, Wu J, Wen N (2018) ADAM17 promotes cell migration and invasion through the integrin beta1 pathway in hepatocellular carcinoma. Exp Cell Res 370(2):373–382. https://doi.org/10.1016/j.yexcr.2018.06.039
51. Yang L, Song X, Zhu J, Li M, Ji Y, Wu F, Chen Y, Cui X, Hu J, Wang L, Cao Y, Wei Y, Zhang W, Li F (2017) Tumor suppressor microRNA-34a inhibits cell migration and invasion by targeting MMP-2/MMP-9/FNDC3B in esophageal squamous cell carcinoma. Int J Oncol 51(1):378–388. https://doi.org/10.3892/ijo.2017.4015
52. Peng X, Zhang Q, Zeng Y, Li J, Wang L, Ai P (2015) Evodiamine inhibits the migration and invasion of nasopharyngeal carcinoma cells in vitro via repressing MMP-2 expression. Cancer Chemother Pharmacol 76(6):1173–1184. https://doi.org/10.1007/s00280-015-2902-9
53. Chen YS, Meng F, Li HL, Liu QH, Hou PF, Bai J, Zheng JN (2016) Dicer suppresses MMP-2-mediated invasion and VEGFA-induced angiogenesis and serves as a promising prognostic biomarker in human clear cell renal cell carcinoma.

Oncotarget 7(51):84299–84313. https://doi.org/10.18632/oncotarget.12520

54. Zhang YE (2017) Non-Smad signaling pathways of the TGF-beta family. Cold Spring Harb Perspect Biol. https://doi.org/10.1101/cshperspect.a022129

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