Periostin silencing suppresses the aggressive phenotype of thyroid carcinoma cells by suppressing the Akt/thyroid stimulating hormone receptor axis

Min Wang · Chunyi Gui · Shenglong Qiu · Jingdong Tang · Zhihai Peng

Received: 17 April 2017 / Accepted: 2 September 2017 / Published online: 30 September 2017
© Springer Science+Business Media B.V. 2017

Abstract Clinical evidence indicates that high periostin expression correlates with aggressive phenotype in thyroid carcinoma. However, the biological roles of periostin in thyroid carcinoma development and progression are still unclear. In this study, we explored the effects of periostin silencing on thyroid carcinoma cell growth, invasion, and tumorigenesis. We also studied the impact of periostin on the activation of phosphoinositide 3-kinase (PI3-K)/Akt signaling, which is involved in the pathogenesis of thyroid carcinoma. It was found that downregulation of periostin significantly inhibited the proliferation, colony formation, and invasion in both FTC-133 and BCPAP thyroid carcinoma cells. In vivo tumorigenic studies confirmed that periostin depletion retarded the growth of subcutaneous FTC-133 xenograft tumors, which was coupled with a significant decline in the percentage of Ki-67-positive proliferating cells.

Western blot analysis demonstrated that periostin downregulation caused a marked inhibition of thyroid stimulating hormone receptor (TSHR) expression and Akt phosphorylation in FTC-133 and BCPAP cells. Co-expression of constitutively active Akt (CA-Akt) significantly reversed periostin-mediated downregulation of TSHR. Most importantly, overexpression of TSHR or CA-Akt rescued FTC-133 cells from periostin-induced growth and invasion suppression. Collectively, periostin regulates thyroid carcinoma growth and progression via the Akt/TSHR axis and represents a promising therapeutic target for this malignancy.

Keywords Akt · Growth · Invasion · Periostin · TSHR

Min Wang and Chunyi Gui have contributed equally to this work.

M. Wang · S. Qiu · J. Tang · Z. Peng (✉)
Department of General Surgery, Shanghai General Hospital, Shanghai Jiaotong University School of Medicine, No. 100 Haining Road, Shanghai 200080, China
e-mail: jimwsy@sina.com

C. Gui
Nursing Department, Eye and ENT Hospital, Fudan University, Shanghai, China

Introduction

Thyroid carcinoma is one of the most frequently detected endocrine neoplasms (Cha and Koo 2016). Although the majority of patients with thyroid carcinoma have a good prognosis after curative resection, some patients (10–15%) have a poor outcome due to early relapse and distant metastasis (Eng et al. 2016; Lim et al. 2016). Thyroid stimulating hormone (TSH) receptor (TSHR) is a member of the G protein-coupled receptor superfamily and acts as a key regulator of thyrocyte growth and function (Kleinau et al. 2017).

Ligation of TSHR by its endogenous ligand TSH contributes to thyroid hormone synthesis and secretion (Davies et al. 2010). TSHR is expressed in both benign and malignant thyrocytes and plays an oncogenic role in thyroid carcinoma (Rowe et al. 2017). It has been reported that TSH enhances cell proliferation in papillary thyroid cancer through TSHR/cAMP/PKA/PAK4 signaling (Xie et al. 2017). Several signaling pathways including phosphoinositide 3-kinase (PI3-K)/Akt signaling have been identified to be involved in the pathogenesis of thyroid carcinoma (Nozhat and Hedayati 2016; Hong et al. 2016). Activation of PI3-K/Akt signaling contributes to interleukin-11-induced epithelial-mesenchymal transition and invasion in anaplastic thyroid carcinoma cells (Zhong et al. 2016). Therefore, the PI3-K/Akt pathway represents an important target for the treatment of thyroid carcinoma.

Periostin is an extracellular matrix protein that plays a pivotal role in bone development, tissue remodeling and repair, and tumorigenesis (Silvers et al. 2016). This gene is aberrantly expressed in numerous cancer types, such as colorectal carcinoma (Xu et al. 2016), head and neck cancer (Qin et al. 2016), and breast cancer (Nuzzo et al. 2016). Induction of periostin expression has been linked to aggressive phenotypes of tumor cells (Xu et al. 2016; Qin et al. 2016). For instance, periostin secreted by glioblastoma stem cells has been reported to recruit tumor-associated macrophages and enhances malignant growth (Zhou et al. 2015). A previous study has documented that periostin is upregulated in papillary thyroid carcinoma and positively correlates with extrathyroidal invasion, distant metastasis, and higher grade staging (Puppin et al. 2008). Another study also demonstrates that upregulation of periostin is significantly associated with extrathyroid invasion, pT and lymph node metastasis in papillary thyroid carcinoma (Bai et al. 2009). Despite these clinical findings, the biological roles of periostin in thyroid carcinoma development and progression are still unknown.

In this work, we examined the functions of periostin in thyroid carcinoma cell growth, invasion, and tumorigenesis. We also checked the impact of periostin on the activation of PI-3 K/Akt signaling.

Materials and methods

Cell culture

Human thyroid carcinoma cell lines (FTC-133, BCPAP, and TPC1) were obtained from the Shanghai Institute for Biological Sciences, Chinese Academy of Science (Shanghai, China) and SW579 cells from American Type Culture Collection (ATCC, Manassas, VA, USA). Nthy-ori 3-1 human thyroid epithelial cells were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS; Sigma-Aldrich) in a humidified incubator at 37 °C with 5% CO₂. For inhibitor experiments, FTC-133 cells were treated with GSK690693 (5 μM; Selleck Chemicals, Houston, TX, USA) for 6, 12, and 24 h and tested for TSHR expression.

Plasmids and transfections

A plasmid expressing periostin-targeting small hairpin RNA (p-shRNA) (sc-61324-SH) or negative control shRNA (c-shRNA) (sc-108060) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The shRNA expressing plasmids contain the puromycin resistance gene. A plasmid expressing a constitutively active isoform of Akt (Kohn et al. 1996) was obtained from Addgene (Cambridge, MA, USA). A fragment containing the entire open reading frame of *TSHR* gene was amplified by PCR using human *TSHR* cDNA (OriGene, Rockville, MD, USA) as a template. The PCR product was purified and inserted into pcDNA3.1(+) vector.

Cells were incubated overnight after plating and transfected with the indicated plasmids using FuGENE 6 Transfection Reagent (Roche Applied Sciences, Mannheim, Germany) as per the manufacturer's instructions. Twenty-four hours after transient transfection, cells were harvested and examined for gene expression, cell viability, and invasion. To obtain stable periostin knockdown subcell lines, FTC-133 and BCPAP cells transfected with periostin-targeting or control shRNA were selected for 7 days in media containing 2 μg/ml puromycin (Sigma-Aldrich).

Quantitative real-time PCR (qRT-PCR) analysis

Cellular RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA). Total RNA was reverse transcribed using the Reverse Transcription System Kit (Promega Corporation, Madison, WI, USA). qRT-PCR was performed using the following primers (Naik et al. 2012): periostin forward, 5'-GCGCTTTAGCACCTTCCT-3' and reverse, 5'-GCACAAATAATGTCCAGTCTCC-3'; GAPDH forward 5'-CGACCACTTTGTCAAGCTCA-3' and reverse, 5'-AGGGGTCTACATGGCAACTG-3'. The relative periostin mRNA level was calculated after normalization to that of GAPDH mRNA.

MTT assay

Cells were seeded onto 96-well culture plates (6×10^3 cells/well) and cultured for 3 or 5 days. The viability of cells was measured using 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay. Cells were incubated with MTT solution (Sigma-Aldrich; 0.5 mg/ml) for 4 h at 37 °C. Dimethyl sulfoxide was added to each well to dissolve the formazan crystals. Absorbance was recorded at 570 nm.

Colony formation assay

Cells were seeded onto 6-well plates (600 cells/well) and allowed to grow for 14 days. Wells were stained with 0.5% crystal violet (Sigma-Aldrich). Colonies consisting of 50 cells or more were counted.

Tumorigenic studies in mice

In vivo tumorigenic studies were performed as described previously (Miao et al. 2016). FTC-133 cells stably expressing periostin or control shRNA (5×10^6 cells/mouse) were inoculated subcutaneously into the flank of 5-week old male athymic nude mice (5 mice for each group). Tumor size was calculated weekly for 5 weeks and growth curves were plotted. After the last measurement, mice were sacrificed and their xenograft tumors were resected, weighed, and photographed. Tumor samples were processed for immunohistochemistry using anti-Ki-67 antibody (ab15580, Abcam, Cambridge, MA, USA; 1:300 dilution) to evaluate cell proliferation. The

percentage of Ki-67-positive cells was determined in 5 microscopic fields for each section (3 sections from each mouse) (Mainetti et al. 2013).

Transwell invasion assay

Cell invasion assay was performed using Transwell chambers (pore size: 8 μ M) that were precoated with Matrigel. Cells were plated into the upper chamber of 24-well plates (2×10^4 cells/well). The lower chamber was filled with DMEM containing 10% FBS. Following 24-h incubation, cells on the upper side of the inserts were removed. The cells that invaded through Matrigel were fixed and stained with crystal violet and counted.

Western blot analysis

Cell lysates were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes using standard protocols. After blocking non-specific binding sites, the membranes were incubated with the following primary antibodies: anti-periostin (ab14041, Abcam; 1:500 dilution), anti-TSHR (ab202960, Abcam; 1:200 dilution), anti-Akt (#9272, Cell Signaling Technology, Danvers, MA, USA; 1:1000 dilution), anti-phospho-Akt (#9271, Cell Signaling Technology; 1:200 dilution), and anti- β -actin (#4967, Cell Signaling Technology; 1:2000 dilution). Horseradish peroxidase-conjugated IgG (Sigma-Aldrich; 1:5000 dilution) was used as secondary antibodies. Protein signals were developed with the ECL chemiluminescence detection kit (Amersham Biosciences, Little Chalfont, UK) and quantified by densitometry using Quantity One (Bio-Rad Laboratories, Hercules, CA, USA).

Statistical analysis

Data are expressed as mean $\pm$ standard deviation and were analyzed using the Student's *t* test or one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. *P* values lower than 0.05 were considered significant.

Results

Knockdown of periostin inhibits cell proliferation and colony formation in vitro

It has been documented that periostin is upregulated in thyroid carcinoma tissues compared to normal thyroid tissues (Puppini et al. 2008). Consistently, we found that periostin was expressed at higher levels in thyroid carcinoma cell lines, especially FTC-133 and BCPAP, than in Nthy-ori 3-1 thyroid epithelial cells ($P < 0.05$; Fig. 1a).

To explore the biological function of periostin in thyroid carcinoma, shRNA-mediated knockdown of

periostin was performed in 2 thyroid carcinoma cell lines (FTC-133 and BCPAP) with relatively high levels of endogenous periostin. Western blot analysis confirmed the downregulation of periostin after transfection with periostin shRNA (Fig. 1b). After culturing for 3 and 5 days, periostin-depleted FTC-133 and BCPAP cells showed a significant reduction of proliferation in comparison with control shRNA-transfected cells ($P < 0.05$; Fig. 1c). Colony formation assay further demonstrated that FTC-133 and BCPAP cells with downregulation of periostin formed significantly fewer colonies than control cells 14 days after low-density plating (Fig. 1d).

Fig. 1 Knockdown of periostin inhibits cell proliferation and colony formation in vitro. **a** Measurement of periostin protein and mRNA levels in 4 thyroid carcinoma cell lines and Nthy-ori 3-1 thyroid epithelial cells by Western blot and real-time PCR analysis, respectively. Numbers below the blots represent fold-change in periostin protein. $*P < 0.05$ versus Nthy-ori 3-1 cells. **b** Western blot analysis of periostin protein levels in FTC-133 and BCPAP cells transfected with control shRNA (c-shRNA) or periostin-targeting shRNA (p-shRNA). Nthy-ori 3-1 cells were

used as a normal control. Numbers below the blots represent fold-change in periostin protein. **c** Cell proliferation assay. FTC-133 and BCPAP cells transfected with c-shRNA or p-shRNA were cultured for 3 and 5 days and tested for viability by MTT assay. $*P < 0.05$. **d** Colony formation assay. FTC-133 and BCPAP cells transfected with c-shRNA or p-shRNA were seeded at a low density and cultured for 14 days to form colonies. $*P < 0.05$

Periostin silencing retards xenograft tumor growth in vivo

Next, we explored whether downregulation of periostin impairs thyroid carcinoma development. To this end, FTC-133 cells stably expressing periostin or control shRNA were inoculated subcutaneously into nude mice and gave rise to xenograft tumors. Downregulation of periostin dramatically retarded the formation of subcutaneous tumors compared to the control group (Fig. 2a). Final tumor weight in the periostin downregulation group was 2.5-fold lower than that in the control group ($P < 0.05$; Fig. 2b). Moreover, immunostaining for the proliferation marker Ki-67 confirmed the lower proliferation capacity of periostin-depleted FTC-133 cells in vivo (Fig. 2c).

Periostin knockdown reduces invasion of thyroid carcinoma cells

Next, we assessed the effect of periostin knockdown on the invasiveness of thyroid carcinoma cells.

Fig. 2 Periostin silencing retards xenograft tumor growth in vivo. FTC-133 cells stably expressing control shRNA (c-shRNA) or periostin-targeting shRNA (p-shRNA) were inoculated subcutaneously into nude mice and tumor growth was monitored. **a** Tumor growth curve was plotted versus time. * $P < 0.05$ between the 2 groups. **b** At 5 weeks after cell

Matrigel invasion assay demonstrated that delivery of periostin shRNA led to 42% reduction of invasion in FTC-133 cells after incubation for 24 h, compared to delivery of control shRNA ($P < 0.05$; Fig. 3a). Similarly, downregulation of periostin significantly decreased the invasive capacity of BCPAP cells by 57% (Fig. 3b). However, the viability of FTC-133 and BCPAP cells was not altered 24 h after transfection with periostin shRNA (Fig. 3c).

Periostin induces TSHR expression through the Akt pathway

Western blot analysis revealed that periostin knockdown led to a 3–5-fold reduction of TSHR expression in FTC-133 and BCPAP cells (Fig. 4a). Moreover, periostin silencing was associated with an inhibition of Akt phosphorylation on Ser473 in thyroid carcinoma cells (Fig. 4a). To check whether periostin regulates the expression of TSHR via activation of Akt signaling, we co-expressed constitutively active Akt in FTC-133 cells with stable downregulation of periostin. As

injection, tumors were resected, photographed, and weighed. **c** Immunostaining for the proliferation marker Ki-67 in the FTC-133 xenograft tumors. The percentage of Ki-67-positive cells was determined. Scale bar = 80 μ m. * $P < 0.05$ versus the c-shRNA group

Fig. 4 Periostin induces TSHR expression through the Akt pathway. **a** Western blot analysis of the indicated proteins in FTC-133 and BCPAP cells after transfection with control shRNA (c-shRNA) or periostin-targeting shRNA (p-shRNA). **b** Western blot analysis of the indicated proteins in FTC-133 cells with stable expression of p-shRNA or c-shRNA after re-transfection with empty vector or constitutively active Akt. **c** FTC-133 cells were treated with GSK690693 (5 μ M) for 6, 12, and 24 h and tested for TSHR expression. Numbers below the blots represent fold-change in protein levels

Moreover, there is a negative correlation between periostin and expression of differentiation markers in papillary thyroid carcinoma (Puppini et al. 2008). In line with the clinical findings, we showed that thyroid carcinoma cells had significantly greater levels of periostin than non-malignant thyroid epithelial cells. The upregulation of periostin may contribute to the aggressive behaviors of cancerous epithelial cells of thyroid carcinoma. Loss-of-function experiments indicated that depletion of periostin significantly suppressed the proliferation and colony formation of FTC-133 and BCPAP cells in vitro and tumorigenesis in vivo. These data underscore the requirement of periostin for thyroid carcinoma growth and also provide a biological explanation for the upregulation of periostin in thyroid carcinoma tissues.

Apart from reduction of proliferation, periostin-depleted thyroid carcinoma cells displayed a significant decline in invasion as determined by Transwell invasion assay. This result is consistent with the clinical association between periostin expression and extrathyroidal invasion in thyroid carcinoma (Puppini et al. 2008). Several previous studies also reported the pro-invasive activity of periostin in different cancer types (Qin et al. 2016; Landré et al. 2016). For

instance, it was found that overexpression of periostin can rescue the invasive phenotype of glioblastoma cells with knockdown of endogenous p73 (Landré et al. 2016). Conversely, silencing of periostin led to an impairment of breast cancer cell motility and invasion (Ishiba et al. 2014). Therefore, periostin is a key player in mediating thyroid carcinoma progression.

Mechanistically, we found that periostin knockdown significantly suppressed the expression of TSHR in thyroid carcinoma cells. Moreover, inhibition of Akt activation abrogated the induction of TSHR expression by periostin, indicating the involvement of Akt signaling in periostin-induced TSHR expression. Periostin has shown the ability to regulate Akt signaling in different cellular contexts (Xu et al. 2015; Xiao et al. 2015). Activation of Akt signaling through induction of integrin-linked kinase 1 is involved in the promotion of endometrial stromal cell migration, invasion, and adhesion by periostin (Xu et al. 2015). Periostin renders colon cancer cells more resistant to chemotherapeutic agents through activation of the PI3 K/Akt/survivin pathway (Xiao et al. 2015). The Akt pathway plays a critical role in thyroid carcinoma progression (Nozhat and Hedayati 2016). Silencing of

Fig. 5 The Akt/TSHR signaling is involved in periostin-mediated aggressiveness. FTC-133 cells with stable expression of periostin-targeting shRNA (p-shRNA) were re-transfected with empty vector or TSHR-expressing plasmid. **a** Western blot analysis was performed to confirm the expression of TSHR protein. Numbers below the blots represent fold-change in TSHR protein. c-shRNA, control shRNA. **b** Cell proliferation assay. The transfected cells were cultured for 3 and 5 days and

tested for proliferation by MTT assay. **c** Transwell invasion assay. The number of cells that invaded through Matrigel-coated inserts was determined after culturing for 24 h. **d** FTC-133 cells with stable expression of periostin-targeting shRNA (p-shRNA) were re-transfected with empty vector or constitutively active Akt. Left, cell proliferation determined by MTT assays. Right, Transwell invasion assay. * $P < 0.05$ versus the vector group

integrin-linked kinase leads to inhibition of Akt phosphorylation in thyroid carcinoma cells, consequently impeding cell migration (Shirley et al. 2016). Activation of PI3 K/Akt signaling is responsible for thyroid carcinoma cell growth and migration evoked by HPIP, a co-repressor for pre-B cell leukemia transcription factor (Wang et al. 2015). TSHR also participates in tumorigenesis of thyroid carcinoma cells (Zheng et al. 2016; Patel et al. 2014). BRAF-activated long noncoding RNA suppresses papillary thyroid carcinoma cell proliferation by downregulating TSHR expression (Zheng et al. 2016). These studies, combined with our data suggest that periostin-

mediated aggressiveness is ascribed to activation of Akt signaling and upregulation of TSHR. In support of this hypothesis, enforced expression of TSHR rescued aggressive phenotype in periostin-depleted thyroid carcinoma cells, as evidenced by increased cell proliferation and invasion. However, it is still unclear to what extent the regulation of thyroid carcinoma growth and invasion by periostin is dependent on the Akt/TSHR pathway.

In conclusion, we provide direct evidence that periostin is required for growth, invasion, and tumorigenesis of thyroid carcinoma cells. The Akt/TSHR axis is involved in periostin-mediated aggressiveness

in thyroid carcinoma. Therefore, targeted reduction of periostin in tumor cells may represent a potential therapeutic strategy against this malignancy.

References

- Bai Y, Kakudo K, Nakamura M, Ozaki T, Li Y, Liu Z, Mori I, Miyauchi A, Zhou G (2009) Loss of cellular polarity/cohesiveness in the invasive front of papillary thyroid carcinoma and periostin expression. *Cancer Lett* 281:188–195
- Cha YJ, Koo JS (2016) Next-generation sequencing in thyroid cancer. *J Trans Med* 14:322
- Davies TF, Yin X, Latif R (2010) The genetics of the thyroid stimulating hormone receptor: history and relevance. *Thyroid* 20:727–736
- Eng OS, Grant SB, Weissler J, Simon M, Roychowdhury S, Davidov T, Trooskin SZ (2016) Operative bed recurrence of thyroid cancer: utility of a preoperative needle localization technique. *Gland Surg* 5:571–575
- Hong S, Yu S, Li J, Yin Y, Liu Y, Zhang Q, Guan H, Li Y, Xiao H (2016) MiR-20b displays tumor-suppressor functions in papillary thyroid carcinoma by regulating the MAPK/ERK signaling pathway. *Thyroid* 26:1733–1743
- Ishiba T, Nagahara M, Nakagawa T, Sato T, Ishikawa T, Uetake H, Sugihara K, Miki Y, Nakanishi A (2014) Periostin suppression induces decorin secretion leading to reduced breast cancer cell motility and invasion. *Sci Rep* 4:7069
- Kleinau G, Worth CL, Kreuchwig A, Biebermann H, Marcinkowski P, Scheerer P, Krause G (2017) Structural-functional features of the thyrotropin receptor: a class A G-protein-coupled receptor at work. *Front Endocrinol (Lausanne)* 8:86
- Kohn AD, Takeuchi F, Roth RA (1996) Akt, a pleckstrin homology domain containing kinase, is activated primarily by phosphorylation. *J Biol Chem* 271:21920–21926
- Landré V, Antonov A, Knight R, Melino G (2016) p73 promotes glioblastoma cell invasion by directly activating POSTN (periostin) expression. *Oncotarget* 7:11785–11802
- Lim ST, Jeon YW, Suh YJ (2016) The prognostic values of preoperative tumor volume and tumor diameter in T1N0 papillary thyroid cancer. *Cancer Res Treat*. doi:10.4143/crt.2016.325
- Mainetti LE, Rico MJ, Fernández-Zenobi MV, Perroud HA, Roggero EA, Rozados VR, Scharovsky OG (2013) Therapeutic efficacy of metronomic chemotherapy with cyclophosphamide and doxorubicin on murine mammary adenocarcinomas. *Ann Oncol* 24:2310–2316
- Miao X, Jia L, Zhou H, Song X, Zhou M, Xu J, Zhao L, Feng X, Zhao Y (2016) miR-4299 mediates the invasive properties and tumorigenicity of human follicular thyroid carcinoma by targeting ST6GALNAC4. *IUBMB Life* 68:136–144
- Naik PK, Bozyk PD, Bentley JK, Popova AP, Birch CM, Wilke CA, Fry CD, White ES, Sisson TH, Tayob N, Carnemolla B, Orecchia P, Flaherty KR, Hershenson MB, Murray S, Martinez FJ, Moore BB, COMET Investigators (2012) Periostin promotes fibrosis and predicts progression in patients with idiopathic pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 303:L1046–L1056
- Nozhat Z, Hedayati M (2016) PI3 K/AKT pathway and its mediators in thyroid carcinomas. *Mol Diagn Ther* 20:13–26
- Nuzzo PV, Rubagotti A, Zinoli L, Salvi S, Boccardo S, Boccardo F (2016) The prognostic value of stromal and epithelial periostin expression in human breast cancer: correlation with clinical pathological features and mortality outcome. *BMC Cancer* 16:95
- Patel PN, Yu XM, Jaskula-Sztul R, Chen H (2014) Hesperetin activates the Notch1 signaling cascade, causes apoptosis, and induces cellular differentiation in anaplastic thyroid cancer. *Ann Surg Oncol* 21:S497–S504
- Puppini C, Fabbro D, Dima M, Di Loreto C, Puxeddu E, Filetti S, Russo D, Damante G (2008) High periostin expression correlates with aggressiveness in papillary thyroid carcinomas. *J Endocrinol* 197:401–408
- Qin X, Yan M, Zhang J, Wang X, Shen Z, Lv Z, Li Z, Wei W, Chen W (2016) TGFβ3-mediated induction of periostin facilitates head and neck cancer growth and is associated with metastasis. *Sci Rep* 6:20587
- Rowe CW, Paul JW, Gedye C, Tolosa JM, Bendinelli C, McGrath S, Smith R (2017) Targeting the TSH receptor in thyroid cancer. *Endocr Relat Cancer* 24:R191–R202
- Shirley LA, McCarty S, Yang MC, Saji M, Zhang X, Phay J, Ringel MD, Chen CS (2016) Integrin-linked kinase affects signaling pathways and migration in thyroid cancer cells and is a potential therapeutic target. *Surgery* 159:163–170
- Silvers CR, Liu YR, Wu CH, Miyamoto H, Messing EM, Lee YF (2016) Identification of extracellular vesicle-borne periostin as a feature of muscle-invasive bladder cancer. *Oncotarget* 7:23335–23345
- Wang SC, Chai DS, Chen CB, Wang ZY, Wang L (2015) HPIP promotes thyroid cancer cell growth, migration and EMT through activating PI3 K/AKT signaling pathway. *Biomed Pharmacother* 75:33–39
- Xiao ZM, Wang XY, Wang AM (2015) Periostin induces chemoresistance in colon cancer cells through activation of the PI3 K/Akt/survivin pathway. *Biotechnol Appl Biochem* 62:401–406
- Xie X, Shi X, Guan H, Guo Q, Fan C, Dong W, Wang G, Li F, Shan Z, Cao L, Teng W (2017) P21-activated kinase 4 involves TSH induced papillary thyroid cancer cell proliferation. *Oncotarget* 8:24882–24891
- Xu X, Zheng Q, Zhang Z, Zhang X, Liu R, Liu P (2015) Periostin enhances migration, invasion, and adhesion of human endometrial stromal cells through integrin-linked kinase 1/Akt signaling pathway. *Reprod Sci* 22:1098–1106
- Xu X, Chang W, Yuan J, Han X, Tan X, Ding Y, Luo Y, Cai H, Liu Y, Gao X, Liu Q, Yu Y, Du Y, Wang H, Ma L, Wang J, Chen K, Ding Y, Fu C, Cao G (2016) Periostin expression in intra-tumoral stromal cells is prognostic and predictive for colorectal carcinoma via creating a cancer-supportive niche. *Oncotarget* 7:798–813
- Zheng H, Wang M, Jiang L, Chu H, Hu J, Ning J, Li B, Wang D, Xu J (2016) BRAF-activated long noncoding RNA modulates papillary thyroid carcinoma cell proliferation through regulating thyroid stimulating hormone receptor. *Cancer Res Treat* 48:698–707

- Zhong Z, Hu Z, Jiang Y, Sun R, Chen X, Chu H, Zeng M, Sun C (2016) Interleukin-11 promotes epithelial-mesenchymal transition in anaplastic thyroid carcinoma cells through PI3 K/Akt/GSK3 β signaling pathway activation. *Oncotarget* 7:59652–59663
- Zhou W, Ke SQ, Huang Z, Flavahan W, Fang X, Paul J, Wu L, Sloan AE, McLendon RE, Li X, Rich JN, Bao S (2015) Periostin secreted by glioblastoma stem cells recruits M2 tumour-associated macrophages and promotes malignant growth. *Nat Cell Biol* 17:170–182