TITLE:
Invasion of uterine cervical squamous cell carcinoma cells is facilitated by locoregional interaction with cancer-associated fibroblasts via activating transforming growth factor-beta

AUTHOR(S):
Nagura, Michikazu

CITATION:
Nagura, Michikazu. Invasion of uterine cervical squamous cell carcinoma cells is facilitated by locoregional interaction with cancer-associated fibroblasts via activating transforming growth factor-beta. 京都大学, 2015, 博士(医学)

ISSUE DATE:
2015-03-23

URL:
https://doi.org/10.14989/doctor.k18894

RIGHT:
Invasion of uterine cervical squamous cell carcinoma cells is facilitated by locoregional interaction with cancer-associated fibroblasts via activating transforming growth factor-beta

Michikazu Nagura a, Noriomi Matsumura a, Tsukasa Baba a,⁎, Ryusuke Murakami a, Budiman Kharma a, Junzo Hamanishi a, Ken Yamaguchi a, Kaoru Abiko a, Masafumi Koshiyama a, Masaki Manda b, Takuya Murata c, Susan K. Murphy d, Ikuo Konishi a

a Department of Gynecology and Obstetrics, Kyoto University Graduate School of Medicine, Japan
b Department of Obstetrics and Gynecology, Kinki University School of Medicine, Japan
c Department of Obstetrics and Gynecology, Kawasaki Medical University, Japan
d Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Duke University Medical Center, Durham, NC, USA

HIGHLIGHTS

• TGF-β pathway is distinguished as a driver initiating invasion of cervical squamous cell carcinoma.
• TGF-β is activated through interaction of squamous cell carcinoma cells and cancer associated fibroblasts at tumor invasive front.

ARTICLE INFO

Article history:
Received 26 August 2014
Accepted 21 November 2014
Available online xxxx

Keywords:
TGF-beta
Invasion
Cancer associated fibroblasts
Cervical cancer

ABSTRACT

Objective. Local invasion is a common pattern of spread in uterine cervical squamous cell carcinoma (CSCC). Although transforming growth factor-beta (TGF-β) facilitates invasion of various types of cancer cells, the role of the TGF-β pathway in CSCC is unclear. In this study, we analyzed the role of TGF-β signaling in the progression of CSCC.

Methods. Immunohistochemistry was used to examine the expression of TGF-β pathway molecules in 67 CSCC samples with clinicopathological data. Activation of the TGF-β pathway was investigated following co-culture of CSCC cells and cervical cancer-associated fibroblasts (CCAFs).

Results. Clinicopathological analysis of CSCC samples revealed that prominent expression of TGF-β receptor-2 was more frequent in CSCC with lymphovascular space invasion (LVSI) than without LVSI (p < 0.01). Lymph node metastasis was more frequent in cases in which phosphorylated SMAD3 (pSMAD3) was localized exclusively at the boundary of tumor clusters (n = 9, p < 0.05). Recombinant TGF-β1 increased pSMAD3 expression and enhanced cellular invasion (p < 0.005) in CSCC cells, which was attenuated by an inhibitor of the TGF-β receptor (p < 0.005). Enhanced pSMAD3 expression and invasion was also observed when conditioned media from CSCC cells co-cultured with CCAFs were administered. Luciferase assays showed that this medium contained a large amount of active TGF-β. Along with TGF-β activation, thrombospondin-1 was upregulated in both CSCC cells and CCAFs, while thrombospondin-1 silencing in either CSCC cells or CCAFs repressed the activity of TGF-β. Thrombospondin-1 was prominently expressed in cases with pSMAD3 boundary staining (p < 0.05).

Conclusions. These results suggest that interaction between CSCC cells and surrounding CCAFs activates TGF-β via thrombospondin-1 secretion to facilitate CSCC invasion.

© 2014 Elsevier Inc. All rights reserved.

Introduction

Despite widespread vaccination against human papilloma virus and periodic cancer screening, cervical cancer remains one of the highest ranking diseases causing mortality in women, and new strategies to treat this disease are urgently needed. Squamous cell carcinoma, the most common histological subtype of cervical cancer, spreads
principally by migrating into the lymphatics or by invading adjacent soft tissues. Eradication of locoregional lesions is critical but is not attainable in cases with tumor extension into the urinary tract and/or rectum for fear of impairing urinary or bowel function. Even if the extension is more limited, it is also difficult to obtain cancer-free margins, leading to pelvic recurrence from residual cancer cells. Obstruction of the ureter(s) is not trivial and is observed in 55.8% of advanced cases [1]. It is, therefore, important to develop effective treatments for invasive extension of this disease, and to this end, it is essential to further elucidate the mechanisms of cervical cancer invasion.

Transforming growth factor-beta (TGF-β) is currently known to promote cancer invasion [2]. TGF-β serves as a potent growth inhibitor for normal epithelial cells [3], but malignant transformed cells acquire resistance to the growth inhibitory effect of TGF-β [4]. Moreover, as breast cancer advances, TGF-β acts as a promoter by inducing epithelial to mesenchymal transition (EMT), invasion, and metastasis [5,6]. In gynecologic cancers, activated TGF-β signaling promotes peritoneal dissemination of ovarian cancer [7]. Thus, TGF-β is widely recognized as a key molecule that drives cancer cell progression, but little is known about the role of TGF-β in uterine cervical cancer.

Growing evidence has shown that the stroma around cancer cells plays an important role in cancer progression. Cancer-associated fibroblasts (CAFs) exhibit morphological phenotypes of myofibroblasts and are known to promote cancer progression through interactions with adjacent cancer cells. TGF-β is a key cytokine mediating such interactions [8,9]. However, we presently lack clarification regarding the role of TGF-β in these interactions in cervical squamous cell carcinoma (CSCC). In this study, we analyze the role of TGF-β signaling in the progression of CSCC. We show that activation of TGF-β-induced by the interaction between CSCC cells and CAFs plays a key role in the initiation of tumor metastasis.

Materials and methods

Tissue samples and immunohistochemistry

Tissue samples and clinicopathological information were collected from sixty-seven patients (median age, 54 +/− 23 years) with stage IB–IIB CSCC who underwent radical hysterectomy or trachelectomy in Kyoto University Hospital from January 2003 to July 2010 with written consent under the approval of the ethics committee. Patient characteristics are described in Table 1.

Table 1. Immunostaining was evaluated for intensity and distribution using the RNeasy Mini Kit (Qiagen, Venlo, Netherlands) and used for quantitative RT-PCR and microarray analysis. Stealth RNA™ siRNA against TSP-1 (Invitrogen, Carlsbad, CA) or Stealth™ RNAi Negative Control Medium GC Duplex (Invitrogen, Carlsbad, CA) was used for RNA interference or non-targeting assays. Cells were transfected with siRNAs by using Lipofectamine 2000 (Invitrogen) in accordance with the manufacturer’s protocol. siRNA sequences were as follows: siTSP-1-1: CCA GAU CAG GCA GAC ACA GAC AAC A/U GUU GUC UGU GUC GUC CUG UAG UGC, siTSP-1-2: CCA CAG GCC AAA GAC GGG UU UAU CAU U/A AUG AAA CCC GUC UUU GGC CUG UGG, and siTSP-1-3: UGG CAU CCC UGA GGC AGA AGA A/U UC UCA UCU GUC GCC UCA GGG AUG CCA.

Cellular functional assays

CSCC cells were treated with/without A83–01 (2 μM) +/− recombinant TGF-β1 (2 ng/ml). Viable cell numbers were determined using a colorimetric assay, and population doubling times were calculated after treatment with TGF-β1. Cellular migration and invasion was assessed by wound healing assays and Boyden-chamber assays as described below.

Wound healing assays

When CSCC cells were 90–100% confluent, “wounds” were introduced by dragging a sterile 1000 μl plastic pipette tip across the cell monolayer. The widths of the scratch lines were measured at five independent locations for each specimen after the treatment. The mean distance filled between the cells most closely spaced on each leading edge of the wound was calculated after 24 h, and the ratio of the distance filled by treated versus untreated cells was designated as the migration value.

Invasion assays

After pretreatment with TGF-β1, CSCC cells were collected and suspended in serum-free medium for Boyden-chamber assays. The
lower chamber was filled with 0.8 ml medium with 10% FBS as a chemotactrant, and in the upper compartment, 8 × 10⁴ cells in 0.2 ml medium were seeded and incubated at 37 °C for 18 h to allow for migration to the lower chamber. Cells that penetrated the membrane coated with BD Matrigel matrix (Becton Dickinson, Franklin Lakes, NJ) were visually counted under light microscopy, and the mean from the sums of five high power fields (×200) was designated as the measure of invasiveness.

Animal studies

Female BALB/cA-Jcl-nu, nu/nu mice were purchased from Nihon Clea (Kyoto, Japan). Animal care and experimental procedures under pathogen-free conditions were performed in accordance with the guidelines of the Institute of Laboratory Animals Graduate School of Medicine, Kyoto University. Subcutaneous xenografts were established in the flanks by inoculating 1 × 10⁶ CaSKi cells carrying the LentitFireTM construct. Lentiviral transduction of target cells was performed with firefly luciferase Luc2 (In Vivo Imaging Solutions, Cheyenne, WY). Thirty-three days later, subcutaneous tumors were harvested, cut into 3 mm pieces, and transplanted into the uterus of 28 independent anesthetized female nude mice to enable development of uterine tumors. Mice with tumor transplants were injected intra-abdominally with A83-01 (150 μg/body, n = 14) or DMSO control (n = 14) three times a week for 58 days. Growth of uterine tumors was tracked once a week using the Xenogen-IVIS Spectrum instrument (PerkinElmer, Waltham, MA) following intra-abdominal injection of D-luciferin (1 mg, Wako, Osaka, Japan). On day 59, remaining mice were euthanized once a week for 58 days. Growth of uterine tumors was tracked once a week for 58 days. Growth of uterine tumors was tracked once a week for 58 days. Growth of uterine tumors was tracked once a week for 58 days. Growth of uterine tumors was tracked once a week for 58 days.

**Co-culture of CSCC cells and CCAFs and TGF-β in conditioned media (CM)**

CSCC cells and CCAFs were plated separately in mono-culture (1 × 10⁴ cells/cm²) or in a 1:1 mixture co-culture (5 × 10⁴ cells/cm², respectively), and the supernatant of cultured media was reserved as “conditioned media” (CM) for further analysis. Tumors were maintained in CM, quantified using the QuantiKin ELISA Human TGF-β1 kit (B&D, Minneapolis, MN). For quantification of the activated form of TGF-β1 in the CM, plasminogen activator inhibitor-1 (PAI-1) promoter/luciferase-transfected mink lung epithelial cells (TLMECs) were used as previously reported [12]. The luminescence of TLMECs was evaluated as the amount of activated TGF-β1 in the CM using the Luciferase Assay System (Promega, Madison, WI).

**Statistical analysis**

In immunohistochemical analyses, Fisher’s exact test was used to test for significant between-group differences. In all other analyses, a two-tailed Student’s t-test was used to test for significance. All statistical analyses were done using GraphPad Prism 5.5 and R software. A p-value < 0.05 was considered statistically significant.

A complete description of the materials and methods, and any associated references are available in Additional supporting information.

**Results**

**Clinicopathological analysis of CSCC in association with the TGF-β pathway**

A total of 67 patients were treated for locally advanced CSCC (IB1; n = 38, IB2; n = 6, IIA1; n = 5, IIA2; n = 2, and IIIb; n = 16). The 67 cases included 22 with lymph node metastasis and two with distant metastasis. Patients with lymphovascular space invasion (LVS1) or lymph node metastasis (LNM) exhibited worse clinical outcomes than those without these characteristics (p < 0.05 for each, Table 1). Immunohistochemical staining was conducted for TGF-β1, TGFBR1, TGFBR2, pSMA3, α-SMA, and TSP1. TGF-β1 was expressed in both tumor cells and stromal areas (Fig. 1A), while TGFBR1 and TGFBR2 were expressed mainly in tumor cells (Fig. 1B–C). The cases with high TGFBR2 expression showed more frequent LVS1 than those with lower expression (p < 0.01, Table 2). Immunostaining of pSMA3 was more definitive. Among 67 cases, pSMA3 expression was undetectable in 34 cases and weakly expressed in another 24 cases, while pSMA3 showed dense staining exclusively in the boundary areas of tumor nests adjacent to stroma in the other nine cases (Fig. 1D). These nine cases with pSMA3 boundary staining more frequently showed lymph node metastasis than the remaining cases (p < 0.05, Table 2). Immunostaining of TSP-1 was found in both tumor and stromal cells (Fig. 1E). In the pSMA3 boundary staining cases with high expression of TSP-1 was observed in 8 of 9 cases, which was more frequent than the other cases (p < 0.05, Table 2). α-SMA was enhanced in stromal areas adjacent to tumor nests (Fig. 1F), indicating that cancer stromal cells had the phenotype of myofibroblasts.

The impact of TGF-β signaling on cellular characters of CSCC cells

Western blotting revealed that pSMA3 expression in CaSki, ME-180, and QG-U cells was induced by recombinant TGF-β1 even at 1 ng/ml, and that pretreatment with A83-01 tempered the induction of pSMA3 (Fig. 2A). SKG-Illa cells did not respond to treatment with recombinant TGF-β1 (data not shown).

Proliferative activity was not altered in CaSki cells by TGF-β1 signal induction with 2 ng/ml recombinant TGF-β1, or by TGF-β signal inhibition with A83-01 (Fig. 2B). Proliferative activity was also not altered in ME-180 cells following treatment with recombinant TGF-β1 or A83-01 (Supplementary Fig. 1A).

Next, in vivo tumor growth assays were conducted to investigate whether inhibition of TGF-β1 pathway signaling could suppress tumor growth or metastasis. After Luc2-transfected CaSki tumor blocks had been transplanted into each uterus of 29 nude mice, the mice were divided into two groups and treated weekly with A83-01 or control solution. Tumor luminescence in each mouse was tracked weekly for nine weeks. There was no significant difference in the luminescent signal, indicating uterine tumors grew with or without A83-01 treatment (Fig. 2C). Six mice treated with A83-01 and one mouse in the control group died prior to day 59, the planned experimental endpoint. Among the euthanized mice, Luc2 detection was prominent in 5′-CAG CTG CAA CCC ATG AA-3′ and 5′-AAC TCG TAC TCG TTG AAG CC-3′.

Please cite this article as: Nagura M, et al, Invasion of uterine cervical squamous cell carcinoma cells is facilitated by locoregional interaction with cancer-associated fibroblasts, Gynecol Oncol (2014), http://dx.doi.org/10.1016/j.ygyno.2014.11.075
Interaction between CSCC cells and cervical cancer-associated fibroblasts (CCAFs)

As shown in Fig. 1F, α-SMA was expressed in stromal areas adjacent to tumor nests, indicating the existence of CCAFs with myofibroblast characteristics. Western blotting showed that α-SMA was expressed in both immortalized cervical cancer-associated fibroblasts (im-CCAFs) and primary cervical cancer-associated fibroblasts (pri-CCAFs), but not in CaSki cells (Fig. 3A).

To investigate whether interactions between CSCC cells and CCAFs influenced the characteristics of CSCC cells, CSCC cells and CCAFs were co-cultured in a 1:1 mixture (henceforth, “CSCC/CCAF”) and we

<table>
<thead>
<tr>
<th>TGF-β1</th>
<th>Staining score: high (&gt;4)</th>
<th>Staining score: low (&lt;=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>38 (57%)</td>
<td>17 (25%)</td>
</tr>
<tr>
<td>Low</td>
<td>7 (10%)</td>
<td>5 (7%)</td>
</tr>
<tr>
<td>p</td>
<td>0.5095</td>
<td>0.086</td>
</tr>
<tr>
<td>TGFBR1</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>High</td>
<td>23 (34%)</td>
<td>4 (6%)</td>
</tr>
<tr>
<td>Low</td>
<td>22 (33%)</td>
<td>8 (12%)</td>
</tr>
<tr>
<td>p</td>
<td>0.4347</td>
<td>0.3029</td>
</tr>
<tr>
<td>TGFBR2</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>High</td>
<td>28 (42%)</td>
<td>6 (9%)</td>
</tr>
<tr>
<td>Low</td>
<td>17 (25%)</td>
<td>16 (24%)</td>
</tr>
<tr>
<td>p</td>
<td>0.0096</td>
<td>0.4372</td>
</tr>
<tr>
<td>pSMAD3</td>
<td>Boundary (+)</td>
<td>Boundary (−)</td>
</tr>
<tr>
<td>Boundary (+)</td>
<td>7 (10%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Boundary (−)</td>
<td>38 (57%)</td>
<td>20 (30%)</td>
</tr>
<tr>
<td>p</td>
<td>0.7066</td>
<td>0.0498</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LVI</th>
<th>+</th>
<th>−</th>
<th>LNM</th>
<th>+</th>
<th>−</th>
<th>TSP-1</th>
<th>High</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF-β1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The effects of TGF-β on CSCC cell behavior. (A) Western blotting revealed that recombinant TGF-β1 (1 or 10 ng/ml) induced pSMAD3 expression in CaSki, ME-180 and QG-U cells, and that A83-01 (1 or 10 μM) reduced this effect. (B) Population doubling time of CaSki cells was not significantly affected by recombinant TGF-β1 and/or A83-01 (n = 8 each). (C) Luciferase activity of uterine tumors derived from luciferase-expressing CaSki cells transplanted into nude mice was not significantly affected by trans-abdominal injection of A83-01. Lucs; relative light units of luminescence of tumor burdens. (D) Recombinant TGF-β1 promoted migration of CaSki cells, which was reduced by A83-01 (n = 5 each). Y-axis; relative migration distance measured in the scratch assay compared with non-treated cells. (E) Recombinant TGF-β1 promoted the invasiveness of CaSki cells, which was reduced by A83-01 (n = 6 each). Y-axis; number of CaSki cells crossing the membrane in invasion assays. (F) Gene Set Enrichment Analysis. “Anastassiou Cancer Mesenchymal Transition Signature” was significantly up-regulated following treatment with recombinant TGF-β1 in CaSki cells (FDRq = 0.006).

To determine which molecule activated TGF-β during the interaction between CSCC cells and CCAFs, gene expression in the mono- and co-cultured CSCC cells and im-CCAFs was examined by quantitative RT-PCR for thrombospondin-1 (TSP-1, Fig. 4A), integrin αv, and neuropilin-1 (Supplementary Fig. 2A). Of these three genes, TSP-1 showed increased expression in CaSki/im-CCAF relative to CaSki and im-CCAF (Fig. 4A, p = 0.0001, p < 0.001, respectively). This up-regulation was most striking on the fourth day of incubation (Supplementary Fig. 2B), and was confirmed on the fourth day at the protein level as well (Fig. 4A). These results were reproduced in ME-180 cells (Fig. 4A).

siRNA-mediated knockdown of TSP-1 expression in either CaSki cells or im-CCAFs was conducted to investigate whether TSP-1 activates the latent form of TGF-β. TSP-1 expression was most prominently reduced by siRNA, “siTSP-1,3” among the three siRNAs tested (Supplementary Fig. 2C). Thus siTSP-1,3 was used for subsequent experiments. Total expression of TSP-1 in CaSki/im-CCAF was markedly repressed, even with TSP-1 knockdown in CaSki cells (Fig. 4B, p < 0.0005) or im-CCAFs (Fig. 4C, p = 0.0005). TMLEC assays consistently showed that the amount of active TGF-β1 in the CM of CaSki/im-CCAF was markedly reduced with TSP-1 knockdown in either CaSki cells (Fig. 4B, p < 0.05) or im-CCAFs (Fig. 4C, p = 0.001).
67 patients, but the nine cases with pSMAD3 boundary staining were not significantly related to the clinical data for the 67 patients, but the nine cases with pSMAD3 boundary staining were more likely to have a high TSP-1 staining score (Table 2, p < 0.05), indicating that TSP-1 activates the TGF-β pathway when SCC cells and CCAFs interact.

Discussion

TGF-β is known to play various roles in malignant progression. It inhibits growth of normal epithelial cells, and conversely, it promotes EMT, invasion and metastasis in cancer cells, thus eluding tumor-suppressive effects [2–4]. With regard to CSCC, the role of TGF-β signaling is more complicated and not well understood. TGF-β pathway functions in an inhibitory manner for pelvic lymph node metastasis [13], while TGF-β1 expression increases through progression from cervical intraepithelial neoplasia to invasive CSCC [14]. Furthermore, TGF-β1 promotes chromosomal instability in human papillomavirus 16 E6E7-infected cervical epithelial cells [15]. Thus in CSCC, TGF-β signaling appears to function in the promotion of tumorigenesis but does not proceed node metastasis although it is known to promote invasion and metastasis in other cancers. In this study, we investigated the role of TGF-β signaling at the initial phase of CSCC progression, which had not previously been well clarified.

We began this study with careful evaluation of the expression status of TGF-β signaling-associated molecules in 67 CSCCs, and revealed that cases with boundary-specific expression of pSMAD3 were more likely to have node metastasis, which was a noteworthy poor prognostic factor. Boundary pSMAD3 expression in tumor clusters was also more frequently accompanied by stromal expression of TGF-β (p < 0.05), which is an activator of TGF-β signaling [16]. These results suggest that to assess the actual tumoral activity resulting from the TGF-β signal TGF-β5 signaling activity in boundary areas of tumor clusters should be investigated, and pSMAD3 boundary staining could be an indicator not only of active TGF-β signaling, but may also serve as a biomarker of progression.

Tumor progression consists of local proliferation and extra-organ spread. The proliferation of the CSCC cell lines was not affected by TGF-β1 or A83-01 both in vitro and in vivo, while TGF-β3 enhanced migration and invasiveness in vitro. GSEA analysis revealed that the gene expression profile of CaSki cells was shifted toward the mesenchymal phenotype by TGF-β3 administration, and the up-regulated genes included FN1, COL5A1, SNAI2 and SOX4 that are known to be involved with EMT [17,18]. These results suggest that TGF-β may not have a role in local proliferation but rather in extra-organ spread of CSCC, resulting in a high rate of node metastasis among the cases with boundary pSMAD3 expression.

There was a correlation between node metastasis (LNM) and boundary pSMAD3 expression, but not between LNM and pSMAD3 expression in whole tumor nests. This result led us to investigate the role of the...
microenvironment on TGF-β1 signal regulation. CAFs surrounding tumor clusters are generally thought to have a myofibroblast phenotype and secrete TGF-β1 to facilitate cancer progression [8,9,11]. The secretion of TGF-β1 was confirmed in CCAFs. Furthermore, when CaSki cells were co-cultured with CCAFs, active TGF-β1 in the CM was increased, and consequently, expression of pSMAD3 was up-regulated and cellular invasion was remarkably augmented. As active TGF-β1 in the CM was also high and pSMAD3 expression increased in breast cancer cells when co-cultured with fibroblasts [19], our results suggest that the amount of active TGF-β1, the biologically relevant form, is regulated during activation through the interaction between CSCC cells and CCAFs.

TGF-β1 is secreted as a latent form and activated by several molecules, such as thrombospondin-1 (TSP-1), integrin αv, and neuropilin-1 [16]. Of these molecules, TSP-1 was markedly up-regulated when CSCC and CCAF cells were co-cultured, and the amount of activated TGF-β1 in the co-cultured CM was reduced when TSP-1 was silenced in either CSCC cells or in CCAF cells. As there was a positive correlation between tumoral TSP-1 expression and pSMAD3 boundary staining, TSP-1 is likely a key factor activating TGF-β1 in CSCC. An increase in TSP-1s is associated with poor survival in liver cancer [20], and the amino-terminal domain of TSP-1 functions to directly promote the invasiveness of papillary thyroid cancer cells [21]. Our results suggest that TSP-1 could increase the invasiveness of CSCC cells through activation of TGF-β1.

In conclusion, we found that interaction between CSCC and stromal cells activates TGF-β1 and that this was mediated by TSP-1 resulting in up-regulation of pSMAD3 in CSCC cells, ultimately promoting invasion. Expression of pSMAD3 in the boundary area of CSCC indicates TGF-β1 activation at the tumor front, and is highly likely to be accompanied by lymph node metastasis. Although we do not yet know what determines distant metastasis via initial invasion, understanding the molecular mechanisms of TGF-β1 activation at the tumor front in cervical carcinoma sheds new light on the mechanism of CSCC progression which will help in developing new treatment strategies.

Conflict of interest statement
All authors declare that they have no conflicts of interest to disclose.

Appendix A. Supplementary data
Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ygyno.2014.11.075.

References


Please cite this article as: Nagura M, et al. Invasion of uterine cervical squamous cell carcinoma cells is facilitated by locoregional interaction with cancer-associated fibroblasts. Gynecol Oncol (2014), http://dx.doi.org/10.1016/j.ygyno.2014.11.075


Todorovic V, Rifkin DB. LTBP5s, more than just an escort service. J Cell Biochem 2012;113:410–8.


