A role for cancer-associated fibroblasts in inducing the epithelial-to-mesenchymal transition in human tongue squamous cell carcinoma

Bin Zhou, Wei-Liang Chen, You-Yuan Wang, Zhao-Yu Lin, Da-Ming Zhang, Song Fan, Jin-Song Li

Department of Oral and Maxillofacial Surgery, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, China

OBJECTIVES: Lymph node metastasis is a prominent clinical feature of tongue squamous cell carcinoma (TSCC) and is associated with a higher mortality rate. Carcinoma-associated fibroblasts (CAFs), a major component of the tumor microenvironment (TME), play an important role in tumor progression, and are associated with a poor prognosis. The aim of this study was to examine the role of CAFs in promoting the invasion of TSCC through the epithelial-to-mesenchymal transition (EMT).

MATERIALS AND METHODS: A series of matched CAF and normal fibroblast (NF) pairs were assessed for cell morphology and for the expression of alpha smooth muscle actin (α-SMA), stromal cell-derived factor-1 (SDF1), fibroblast-activating protein (FAP), vimentin, and cytokeratin (CK) markers. Transwell assays, Western blot analysis, reverse transcription-PCR, and immunofluorescence staining were used to assess the role of CAFs, as compared to that of NFs, in promoting proliferation, migration, invasion, and EMT in TSCC.

RESULTS: Both CAF and NF primary cultures expressed vimentin but not CK. CAFs showed significantly higher α-SMA protein levels, SDF1 secretion, and mRNA levels of α-SMA, SDF1, and FAP. We also found that co-culture with CAFs enhanced the proliferation and invasion of SCC9 cells. Moreover, co-culture with CAFs induced upregulation of the EMT markers fibronectin and vimentin, downregulation of E-cadherin, and enhanced invasion in SCC9 cells.

CONCLUSION: These results suggest that CAFs induce EMT marker expression and functional changes in TSCCs.

Keywords: cancer-associated fibroblasts; epithelial-mesenchymal transition; tongue squamous cancer cells; tumor microenvironment

Introduction

Tongue squamous cell carcinoma (TSCC) is the most common oral cancer and frequently leads to malfunctions in mastication, speech, and deglutition (1). Despite the advances in oral cancer therapeutics, the 5-year survival rate remains stubbornly low. Traditionally, treatments for TSCC have focused on the tumor cells. However, increasing studies have demonstrated that cancer, a vastly complex entity, also involves various types of mesenchymal cells and the extracellular matrix, collectively termed the tumor microenvironment (TME) (2–5). Cancer cells recruit benign microenvironmental cells, which constitute a major component of the microenvironment, to facilitate cancer cell growth, survival, invasion, and dissemination (3), providing an alluring alternative to traditional tumor-cell-targeted therapy.

As the most abundant component of TME, cancer-associated fibroblasts (CAFs) are widely known to be co-conspirators in tumor initiation and progression. CAFs acquire a phenotype similar to myofibroblasts (MFs), which are activated in wound healing and fibrosis and possess a different morphology and function from normal fibroblasts (6). Unlike the MFs removed by apoptosis in normal wound healing, CAFs are involved in carcinogenesis and in the multifaceted mechanisms of cancer progression, including extracellular matrix remodeling, immune suppression, and secretion of the growth factors and cytokines that extensively affect tumor cell growth, invasion, differentiation, angiogenesis, and chronic inflammation (6, 7). A CAF-rich reactive stroma is associated with high-grade malignancies and poor prognoses in oral cancer (8–11), breast cancer (12), colorectal cancer (13), and several other cancers.

As with many other solid tumors, metastatic disease, especially that in the lymph nodes, is the most important prognostic factor in oral patients with cancer. Cancer cells can invade as single or collective cell migrations that retain
epithelial characteristics (3). Epithelial-mesenchymal transition (EMT), a process in which epithelial cells lose cell–cell contact and develop mesenchymal properties, is thought to enhance motility, invasion, and apoptosis resistance (14–16). In patients with cancer, the expression of EMT inducers, such as Snail and Slug, has been correlated with a higher tumor grade and a worse prognosis (14). Vered et al. reported that EMT markers commonly expressed in both primary and metastatic oral cancers and cancer cells with decreased E-cadherin expression are primarily located at the tumor periphery and directly contact CAFs, revealing that EMT may be modulated by CAFs (17). We thus hypothesize that CAF primary cultures are important for invasion and EMT potential in TSCC.

In this study, we established 16 sets of patient-matched primary fibroblast cultures from resected tongue cancers. The characteristics and properties that promote cellular proliferation and invasion in SCC9 were compared between CAFs and NFs; conditioned medium and co-culture models were established to investigate the role of CAFs in EMT induction in SCC9.

**Materials and methods**

**Cell culture**

The human tongue cancer cell line SCC9 was obtained from the American Type Culture Collection and maintained in DMEM/F12 (Hyclone, Logan, UT, USA) with 10% fetal bovine serum (FBS, Hyclone, USA) at 37°C and 5% CO2.

Tongue tissues were collected from 16 patients with TSCC who underwent surgical resection at Sun Yat-sen Memorial Hospital, Guangzhou, China. The study was approved by the Human Research Ethics Committee at Sun Yat-sen Memorial Hospital. Written informed consent was obtained from patients after full explanation of the study purpose and procedures. Both the tumor and corresponding normal tongue parenchyma, which was at least 2 cm from the macroscopic tumor margin, were used to establish primary CAF and NF cultures. Harvested tissues were minced and digested at 37°C by rotating in medium containing 0.2% collagenase II (Gibco, Rockville, MD, USA) for 2 h. After centrifugation, these tissues were minced and digested at 37°C containing 0.2% collagenase II (Gibco, Rockville, MD, USA) with shaking for another 30 min. The cells were passed through a 70-μm filter (BD Bioscience, Franklin Lakes, NJ, USA) and cultured in DMEM/F12 supplemented with 10% FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin for 1–2 weeks. After differential trypsinization for 3 min at 37°C, purified fibroblasts were separated from epithelial cells. The primary cell cultures were used before their 10th passage for all experiments in this study.

**Pathological review**

Representative hematoxylin and eosin (H&E)-stained slides of all 16 cancer samples, of which four patients were recurrent, were independently acquired and reviewed. Histologic subtypes and stage were determined according to the guidelines of World Health Organization and the 2010 criteria of the International Union Against Cancer, respectively (18).

**Conditioned medium experiments**

The conditioned medium from primary fibroblasts grown in DMEM/F12 supplemented with 10% FBS for 48 h was collected. SCC9 cells were seeded in six-well plates, and after 24 h, incubated with a 1:2 mixture of fibroblast conditioned and fresh 10% FBS DMEM/F12 media for 2 weeks.

**Indirect co-culture of fibroblasts with cancer cells**

SCC9 cells were seeded in six-well plates, and fibroblasts were seeded on the 0.4-mm polyester membrane of a 12-mm transwell insert (Corning, New York, NY, USA) and placed in a separate culture plate. The next day, the transwell insert was transferred to the culture plate containing the SCC9 cultures, and the SCC9 and fibroblasts were co-cultured for 2 weeks. The media were replenished after 3 days of starting the co-culture.

**Quantitative RT-PCR (qPCR)**

Total RNA was extracted from cells using TRIzol (Invitrogen, Carlsbad, CA, USA). Total RNA (1 μg) was reverse transcribed into cDNA using an oligo (dT) primer and then amplified using the primer sets specified in Table 1 following the manufacturer’s protocols. The expression of α-SMA, SDF1, FAP, E-cadherin, fibronectin, and vimentin was normalized to β-actin, and the expression of the control sample was set to a value of one.

**Western blot analysis**

The cell lysates were prepared as described previously (1). Equal amounts of protein samples were resolved by 8% SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (BioRad, Berkeley, CA, USA). The membranes were blocked with 5% non-fat milk at room temperature, probed overnight with the primary antibody at 4°C and then with the peroxidase-conjugated secondary antibody (ProteinTech, Chicago, IL, USA), and visualized with chemiluminescence (GE, Fairfield, CT).

### Table 1  Sequences of primers used for qPCR

<table>
<thead>
<tr>
<th>Sense (5’–3’)</th>
<th>Anti-sense (5’–3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-SMA</td>
<td>GCAGCCCCAGCAAGCAGCTGT</td>
</tr>
<tr>
<td>SDF1</td>
<td>TCCTTGTGAGTGTGTTTT</td>
</tr>
<tr>
<td>FAP</td>
<td>TCTAAGGGAAAGAGATGGCACAA</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>GGAGGAGGAGGGGTGTTCAAA</td>
</tr>
<tr>
<td>Vimentin</td>
<td>GACACTGCTCTGGGAACGTCTT</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>CAGTGGGGAGACCTCGAGAAG</td>
</tr>
<tr>
<td>β-actin</td>
<td>AGCCTCGCTTTGGCCGATCC</td>
</tr>
</tbody>
</table>
USA). Quantitation of the protein bands was performed using the IMAGE J software (NIH, Bethesda, MD, USA).

**Enzyme-linked immunosorbent assay (ELISA)**

Fibroblasts \((1 \times 10^7$/well\)) were plated in six-well culture plates and grown in serum-free DMEM for 72 h. Conditioned medium was collected and assayed for SDF1 using an SDF1 ELISA kit (R&D Systems, Minneapolis, MN, USA) following the manufacturer’s instructions. Each experiment was assayed in duplicate and repeated at least three times.

**Immunofluorescence staining**

Fibroblasts and SCC9 were subjected to immunofluorescence staining on coverslips. After fixation and permeabilization, the cells were incubated with primary antibodies against α-SMA (R&D Systems), SDF1 (Abcam, Cambridge, MA, USA), vimentin, E-cadherin, fibronectin (Santa Cruz, Santa Cruz, CA, USA), and cytokeratin (ZSGB, Beijing, Beijing, China), followed by FITC-conjugated secondary antibodies (Invitrogen). The coverslips were counterstained with 4-diamidino-2-phenylindole and imaged using the TCS SP5 Confocal Microscope (Lecia, Solms, Germany).

**MTT assay**

In 96-well plates, SCC9 cells were cultured with conditioned media derived from 3-day-old fibroblast cultures and then incubated with 10 μl MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] for 60 min. The culture media were subsequently removed, and the resulting crystals dissolved with 100 μl DMSO by shaking for 10 min. The optical absorbance (OD) was immediately measured at 490 nm using a microplate reader. The relative cell growth was calculated as \((OD \text{ CAFs} / OD \text{ NFs})\%\).

**Invasion assay**

Approximately \(0.5 \times 10^4\) fibroblasts were placed in the lower chamber of a polycarbonate transwell filter chamber (Corning, USA) overnight in 400 μl serum-free F12/DMEM, after which \(1 \times 10^4\) SCC9 cells were plated in the upper chamber. The cells were incubated for 12 h at 37°C. The cells on the lower membrane surface were fixed in 4% paraformaldehyde for 15 min, stained with 0.05% crystal violet for 15 min, and counted (five random fields (100 × magnification) per well).

**Statistical analysis**

Data were expressed as the means ± standard deviation (SD). The normal distribution of the data was assessed using the Shapiro–Wilk test, and statistical analyses between two groups were performed using the non-parametric Mann–Whitney U-test or the parametric Student’s t-test. A value of \(P < 0.05\) was considered to indicate statistical significance. All statistical analyses were performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

**Results**

Isolation of CAFs and NFs

Sixteen matched pairs of CAFs and NFs were successfully established from fresh human tongue cancer specimens after surgical resection. The patient demographics and pathology details of the tongue cancer samples are shown in Table 2. Phase-contrast microscopy revealed similar spindle-like appearances in both CAFs and NFs (Fig. 1, left). To exclude the possibility of epithelial cell contamination, expression of cytokeratin and vimentin was evaluated in the fibroblasts. The CAF and NF cell populations positively stained for vimentin, which is normally expressed in fibroblasts but not epithelial cells, whereas the cells did not stain for cytokeratin, which is specific to epithelial cells (Fig. 1, middle and right). These results suggest that the primary cultures were uniformly composed of fibroblasts.

**Differential characteristics between CAFs and NFs**

To characterize the CAFs and NFs, we first evaluated the expression of α-SMA, a well-recognized marker for CAFs (19), in five matched CAF and NF pairs. Using Western blot analysis, we found that the expression of α-SMA in CAFs was significantly higher than that in the matched NFs.

<table>
<thead>
<tr>
<th>CAFs/NFs cell line</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Size (mm)</th>
<th>Stage (TNM)</th>
<th>Clinical staging</th>
<th>Operation</th>
<th>Type of cancer</th>
<th>Differentiated degree</th>
<th>Recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>25</td>
<td>30</td>
<td>T2N0M0</td>
<td>II</td>
<td>TR, SND</td>
<td>SCC</td>
<td>Well</td>
<td>N</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>68</td>
<td>50</td>
<td>T4N2M0</td>
<td>IV</td>
<td>TR, RND</td>
<td>SCC</td>
<td>Well</td>
<td>N</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>61</td>
<td>28</td>
<td>T2N0M0</td>
<td>II</td>
<td>TR, SND</td>
<td>SCC</td>
<td>Poorly</td>
<td>Y</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>28</td>
<td>35</td>
<td>T2N0M0</td>
<td>II</td>
<td>TR, SND</td>
<td>SCC</td>
<td>Well</td>
<td>N</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>62</td>
<td>45</td>
<td>T3N1M0</td>
<td>III</td>
<td>TR, RND</td>
<td>SCC</td>
<td>Well</td>
<td>N</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>47</td>
<td>15</td>
<td>T1N0M0</td>
<td>I</td>
<td>TR, SND</td>
<td>SCC</td>
<td>Well</td>
<td>N</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>40</td>
<td>18</td>
<td>T1N0M0</td>
<td>I</td>
<td>TR, SND</td>
<td>SCC</td>
<td>Moderately</td>
<td>N</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>46</td>
<td>42</td>
<td>T3N0M0</td>
<td>III</td>
<td>TR, RND</td>
<td>SCC</td>
<td>Poorly</td>
<td>N</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>45</td>
<td>48</td>
<td>T4N1M0</td>
<td>IV</td>
<td>TR, RND</td>
<td>SCC</td>
<td>Moderately</td>
<td>Y</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>54</td>
<td>32</td>
<td>T2N0M0</td>
<td>II</td>
<td>TR, RND</td>
<td>SCC</td>
<td>Moderately</td>
<td>N</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>56</td>
<td>15</td>
<td>T1N0M0</td>
<td>I</td>
<td>TR, SND</td>
<td>SCC</td>
<td>Well</td>
<td>N</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>34</td>
<td>43</td>
<td>T3N0M0</td>
<td>III</td>
<td>TR, RND</td>
<td>SCC</td>
<td>Moderately</td>
<td>N</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>47</td>
<td>45</td>
<td>T4N2M0</td>
<td>IV</td>
<td>TR, RND</td>
<td>SCC</td>
<td>Well</td>
<td>Y</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>43</td>
<td>48</td>
<td>T4N1M0</td>
<td>IV</td>
<td>TR, RND</td>
<td>SCC</td>
<td>Well</td>
<td>N</td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>58</td>
<td>28</td>
<td>T2N0M0</td>
<td>II</td>
<td>TR, SND</td>
<td>SCC</td>
<td>Moderately</td>
<td>Y</td>
</tr>
</tbody>
</table>

F, female; M, male; N, no; Y, yes; SCC, squamous cell carcinoma; TR, tumor resection; RND, radical neck dissection; SND, supraomohyoid neck dissection.
Using qPCR, the quantification of α-SMA mRNA levels revealed significant differences among all CAF-NF pairs, ranging from 1.73 ± 0.11- to 9.37 ± 0.66-fold higher α-SMA expression in CAFs than NFs (Fig. 2B), which was consistent with the observed protein levels. We next examined SDF1 secretion, which promotes migration and invasion in cancer cells (20). As shown in Fig. 3A and B, there was a 4.01 ± 1.20-fold increase in secreted SDF1 levels in CAFs relative to the corresponding NFs, as measured by ELISA, and a 5.13 ± 4.44-fold increase in mRNA levels, as measured by qPCR. In addition, there was a significant increase (2.23 ± 0.65-fold) in FAP mRNA levels in CAFs relative to NFs (Fig. 3D). Immunofluorescence images confirmed the increase in α-SMA and SDF1 protein levels in CAFs (Fig. 2C and 3C).

CAFs regulated proliferation and invasion in SCC9 cells
To elucidate a link between CAFs and cancer cells, we evaluated the effect of CAF conditioned medium on the proliferation of SCC9 cells. MTT assays showed that SCC9 cells cultured in medium conditioned by CAFs, compared with that of NFs, exhibited a 1.22 ± 0.05-fold increase in cell density after 3 days (Fig. 4A). Similarly, we examined the migration of SCC9 cells using a transwell assay. As shown in Fig. 4B, CAFs induced a significantly higher invasion potential in SCC9 cells than in NFs.

CAFs induced EMT in SCC9 cells
We next hypothesized that EMT is involved in the CAF-stimulated invasion in SCC9 cells. We co-cultured SCC9 cells with CAFs for 2 weeks both in an indirect co-culture system and in a conditioned medium experiment. Stimulation of SCC9 cells with CAFs, as compared to matched NFs, decreased the protein levels of E-cadherin and markedly increased the levels of several well-characterized mesenchymal markers, including fibronectin and vimentin, as shown by Western blotting (Fig. 5A). The observed mRNA changes in SCC9 cells were also in accordance with EMT (Fig. 6A–C). Immunofluorescence staining showed that E-cadherin levels were decreased and fibronectin and vimentin levels were increased in SCC9 cells co-cultured with CAFs, but not with NFs (Fig. 5B).

CAFs increased the invasion potential in SCC9 cells
To determine whether the changes in EMT markers in the SCC9 cells co-cultured with CAFs were linked to altered functions in the cancer cells, we measured their invasion potential. The invasion potential of SCC9 cells co-cultured with CAFs or without fibroblasts was much lower than when co-cultured with CAFs, in which case the SCC9 cells acquired change characteristic of EMT (Fig. 6D).

Discussion
Tongue squamous cell carcinoma, a complex tissue comprising tumor cells and several other types of TME non-
malignant cells, is characterized by a greater migration potential and more aggressive behavior than that of other oral cancers (1, 20). The tumor-supporting cells are recruited by the cancer cells to enhance their survival, growth, invasion, and dissemination (3). In recent years, elucidation of the underlying molecular mechanisms and the cancer cell–TME relationship has implicated alterations in various pathways. This valuable information is currently being exploited to develop prospective treatment protocols.

Figure 3  (A) SDF1 levels were measured by ELISA in matched CAFs and NFs. CAFs secreted an average of 4.01 ± 1.20-fold more SDF1 compared with matched NFs ($P < 0.01$). Representative data from three independent experiments are shown. (B) qPCR showed that CAFs expressed significantly higher SDF1 than did matched NFs. $P < 0.05$ marked with *, and $P < 0.01$ marked with #. Representative data from three independent experiments are shown. (C) Immunofluorescence staining for SDF1 in CAFs and NFs. Nuclei: blue. Scale bar: 10 µm. (D) qPCR showed that CAFs expressed significantly higher FAP than did matched NFs. $P < 0.05$ marked with *, and $P < 0.01$ marked with #, but $P = 0.14$ for CAF/NF2.

Figure 4  (A) MTT assays displayed an increased proliferative capacity in SCC9 cells after 3 days of culture in CAF conditioned media ($P < 0.001$). Representative data from three independent experiments are shown. (B, C) Modified Boyden chamber assays demonstrated that CAFs, compared with NFs and the control, enhanced invasion in SCC9 cells (100 × magnification). Representative data from three independent experiments are shown.
It has been reported that CAFs are involved in tumor initiation and progression. CAFs potentially derive from several types of cells, such as pre-existing resident fibroblasts, nearby epithelial cells via EMT, bone marrow-derived cells, and endothelial cells (7, 21, 22). However, the definition of CAFs remains ambiguous. Morphological

<table>
<thead>
<tr>
<th></th>
<th>SCC9 + Control</th>
<th>SCC9 + CAFs</th>
<th>SCC9 + NFs</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-cad</td>
<td>2</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>FN</td>
<td>2</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Vim</td>
<td>2</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>GAPDH</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 5** (A) Western blot analysis of mesenchymal markers (vimentin and fibronectin) and an epithelial marker (E-cadherin) in SCC9 cells cultured in CAF or NF conditioned medium for 2 weeks. Untreated SCC9 cells were used as the control. (B) Immunofluorescence staining illustrated reduced expression of E-cadherin and increased expression of vimentin and fibronectin in SCC9 cells cultured with CAFs. Nuclei: blue. Scale bar: 10 μm. Representative data from three independent experiments with different CAF strains from three patients are shown.

**Figure 6** (A, B, and C) qPCR showed that SCC9 cells cultured in CAF conditioned media expressed significantly lower E-cadherin but markedly higher fibronectin and vimentin, as compared to SCC9 cells cultured with matched NF conditioned media (P < 0.01). Representative data from three independent experiments with different CAF strains from three patients are shown. (D) Invasion assays demonstrated that SCC9 cells cultured in CAF conditioned media were more invasive than those cultured in NF conditioned or unconditioned media (100 × magnification). Representative data from three independent experiments are shown.
differences between CAFs and NFs have not been investigated extensively, aside from studies in tongue cancer CAFs, which revealed that (i) CAFs are long spindle-shaped cells with small cytoplasmic protrusions, (ii) CAFs are variable in size, and (iii) CAFs display a crowded, disordered arrangement comprising overlapping cells that have lost contact/density inhibition (23). Several mesenchymal markers, such as α-SMA, FAP, and vimentin, are expressed in activated fibroblasts (24). CAFs are typically identified by expression of α-SMA (10, 19, 25). Our study also successfully confirmed the differential expression of α-SMA among matched CAFs and NFs. High secretion of SDF1 could also be a characteristic of CAFs. Unlike α-SMA, a cancer cell marker without any known special function, SDF1 has been shown to promote invasion in breast (26) and ovarian (27) cancers and to induce EMT in OSCC (28). FAP, another marker strongly implicated by several other studies (24, 29), was confirmed here to also be upregulated at the mRNA level. These results provide more information that will contribute to definition of CAFs.

Carcinoma-associated fibroblasts play a critical role in tumor–stromal interactions. On the one hand, CAFs promote the proliferation and invasion of cancer cells through signals, such as growth factors and chemokines. Previous reports have shown that CAFs can stimulate tumorigenesis, proliferation, invasion, and metastasis of cancer cells by secreting high levels of keratinocyte growth factor (30), actin A (31), monocyte chemotactic protein-1 (32), endothelin-1 (33), chemokine (C-C motif) ligand 7 (CCL7/MCP-3) (34) in oral cancer, palladin (35) in pancreatic cancer, and hepatocyte growth factor (36) in gastric cancer, although different CAF subtypes have differential tumor-promoting capability (37).

On the other hand, cancer cells can influence the activation and function of fibroblasts by secreting cytokines, such as TGFβ1 (38, 39) and EMMPRIN/CD147 (40). However, the abovementioned findings are all based on indirect co-culture systems. Flåberg et al. (41, 42) investigated the effects of fibroblasts derived from normal subjects and patients with tumor on the growth of tumor cells using direct cell-to-cell contact studies in vitro; they found that most of the fibroblasts inhibited proliferation of the tumor cells and that effective inhibition required the formation of a morphologically intact fibroblast monolayer. Yet, co-implantation tumor xenograft models demonstrated that CAFs extracted from human breast carcinomas promoted the growth of admixed breast carcinoma cells to a significantly greater degree than did matched NFs (26). In our present study, the proliferation and invasion potential of SCC9 cells were dramatically increased by CAF stimulation; these results support our previous assumption that CAFs positively regulate cancer cells.

In present studies, CAFs have been reported to induce EMT and enhance the migration potential of cancer cells. Giannoni et al. (24) investigated the effects of human prostate CAF conditioned medium on PC3 cells and demonstrated evident EMT in PC3, as well as enhanced tumor growth and metastases through metalloproteinase secretion. Similar results were reported in the PMC42-LA (43) and MCF7 (44) breast cancer cell lines. In oral cancer, CAFs initiated EMT in SCC25 cells via increased production of BDNF, which interacted with upregulated TrkB expressed on the surface of EMT-converted SCC25 cells (20). However, these CAFs were induced by co-cultured fibroblasts and SCC25. Our study further demonstrated that after 2 weeks of co-culturing, primary CAF cultures, compared with five matched NF pairs, promoted the transition and invasion of SCC9 cells. Furthermore, the results of the conditioned medium experiment were consistent with that of the co-culture system. This suggests that despite the potentially different origins and heterogeneity of CAFs, the cells appeared to share similar characteristics. Considering the significantly increased SDF1 secretion in CAFs and the stimulatory capabilities of SDF1, we further hypothesize that CAFs promote proliferation, invasion, and EMT in cancer cells through secretion of SDF1; CAF-derived SDF1 may also be involved in the proliferation of cancer stem cells, such as CD44+/CD24− cells (45), which represent a potentially important therapeutic target.

In conclusion, human tongue cancer CAFs differed from NFs with respect to α-SMA expression, SDF1 secretion, and the induction of proliferation and invasion in cancer cells. Moreover, our findings on the stimulatory effect of CAFs on EMT and invasion in TSCC may provide insight into the malignant progression of human TSCC, which should be considered during treatment of tongue cancer.

References


Acknowledgement

This work was supported by the National Natural Science Foundation of China (No.81172563, to Wei-liang Chen) and a research grant from the Guangzhou Science and Technology Foundation (No.11C32060743, to Wei-liang Chen).

Conflict of interest

None declared.
学霸图书馆
www.xuebalib.com

本文献由“学霸图书馆-文献云下载”收集自网络，仅供学习交流使用。

学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，提供一站式文献检索和下载服务”的24小时在线不限IP图书馆。

图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：
图书馆首页 文献云下载 图书馆入口 外文数据库大全 疑难文献辅助工具