Cancer-associated fibroblast stimulates cancer cell invasion in an interleukin-1 receptor (IL-1R)-dependent manner

XIANGLAN ZHANG[1,2] and YOUNG SUN HWANG[3]

[1]Department of Pathology, Yanbian University Hospital, Yanji, Jilin 133000, P.R. China; [2]Oral Cancer Institute, Yonsei University College of Dentistry, Seoul 03722; [3]Department of Dental Hygiene, College of Health Science, Eulji University, Seongnam, Gyunggi-Do 13135, Republic of Korea

Received May 3, 2019; Accepted August 5, 2019

DOI: 10.3892/ol.2019.10784

Abstract. Tumor microenvironment serves an important role in tumor growth and metastasis. Cancer cells can promote growth and malignancy by altering the surrounding stroma. Cancer-associated fibroblast (CAF) are an abundant cell type present within the tumor microenvironment and provide tumorigenic features by secreting cytokines. In the current study, the CAF-mediated invasion of oral squamous cell carcinoma (OSCC) was investigated and the associated mechanisms were elucidated. Cancer invasion was estimated using a Matrigel-coated Transwell chamber and FITC-gelatin matrix. To verify the effect of the tumor microenvironment, conditioned media (CM) from normal fibroblast (NF) and CAFs were prepared. An ELISA was performed to estimate the level of IL-1β. A proteome profiler human protease array was performed to verify the proteases affected by stimulation with CM, from CAF. Recombinant IL-1β protein increased the invasion of OSCC cells. IL-1β expression was higher in CAF than NF. CM from CAF (CM-CAF) increased cancer invasion and FITC-gelatin matrix degradation. The invasive capacity provided by CAF was abrogated by an IL-1 receptor (IL-1R) antagonist. Additionally, CM-CAF increased the secretion of ADAM 9 and Kallikrein 11 from OSCC cells. The invasion activity by CM-CAF was partially abrogated by the neutralization of ADAM 9 or Kallikrein 11. In conclusion, by providing stromal factor, CAFs were a critical inducer of OSCC invasion, and CAF secretes the required amount of IL-1β to increase cancer invasion activity. The invasive capacity of CAF was identified to be IL-1R-dependent. ADAM 9 and Kallikrein 11 were influencing factors involved in the increase of CAF-mediated cancer invasion.

Correspondence to: Dr Young Sun Hwang, Department of Dental Hygiene, College of Health Science, Eulji University, 553 Sansung-Daero, Soojung-Gu, Seongnam, Gyunggi-Do 13135, Republic of Korea
E-mail: kiteys@eulji.ac.kr

Abbreviations: OSCC, oral squamous cell carcinoma; CM, conditioned media; CAF, cancer-associated fibroblast; ELISA, enzyme-linked immunosorbent assay

Key words: tumor microenvironment, cancer-associated fibroblast, interleukin-1β, invasion, protease

Introduction

The tumor microenvironment is closely related to cancer initiation, progression, and invasion (1). The tumor microenvironment consists of extracellular matrix (ECM), stromal cells (such as fibroblasts, myofibroblasts, neuroendocrine cells, adipose cells, immune and inflammatory cells, blood, and lymphatic vascular networks) and immune cells (including T and B lymphocytes, natural killer cells, and tumor-associated macrophages), however, the precise function of each constituent remains unknown (2). Tumor initiation proceeds with a complex series of biological changes whereby normal cells acquire uncontrolled cell growth and resistance to cell death. In conditions of hypoxia, oxidative stress, and acidosis, the tumor microenvironment alters cellular metabolism and leads to the subsequent evolution of malignancies. The overexpression of oncogenes during tumor growth and progression by stromal stimuli can affect the aggressiveness of cancer. Cancer-associated fibroblasts (CAFs) were shown to promote tumor growth and the invasion of low-invasive cancer cells in xenografted mice (3). Therefore, a complex tissue microenvironment is necessary for tumor progression and metastasis (4,5). Research on the tumor microenvironment is important to identify therapeutic targets and for the diagnosis of cancer.

Cancer cells that have acquired the ability to invade infiltrate nearby healthy tissues and spread beyond the tissue layer. Reports that many kinds of cytokines and chemokines were involved in every stage of tumorigenesis have suggested that reciprocal cross-talk between cancer and adjacent stroma is important for cancer progression (6). The role of platelet-derived growth factor (PDGF), fibroblast activation protein (FAP), fibroblast growth factor receptor (FGFR), vitamin D receptor (VDR), transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), interleukin-10 (IL-10), interleukin-12 (IL-12), epidermal growth factor (EGF), and vascular endothelial growth factor (VEGF) have been suggested as critical tumor microenvironment factors for

tumor progression (1). In addition, matrix metalloprotease-2 (MMP-2) from cancer-associated fibroblasts has been reported to induce epithelial invasion and dis-cohesion of keratinocytes into collagen (7). Therefore, analysis of cross-talk between cancer and CAF is important in understanding the mechanism of cancer invasion.

In this study, we observed the role of CAFs in cancer invasion as tumor microenvironment and elucidated the related mechanism. Our results demonstrated that CAFs were critical inducers of OSCC invasion and that their invasive capacity was IL-1R-dependent. CAF stimulation results in an increase in protease, such as ADAM 9 and Kallikrein 11. The results of this study may be useful in helping to identify the role of CAFs in cancer invasion and progression and to generate therapeutic strategies for treating cancers.

Materials and methods

Cell culture. HSC-2 oral squamous cell carcinoma (OSCC) cells was grown in DMEM/F12 (3:1 ratio) medium supplemented with 10% FBS, $1x10^{-10}$ M cholera toxin, 0.4 mg/ml hydrocortisone, 5 μg/ml insulin, 5 μg/ml apo-transferrin, and $2x10^{-11}$ M triiodothronine (T3) in a humidified atmosphere of 5% CO_2 at 37˚C. Normal gingival fibroblasts (NF) and cancer-associated fibroblasts (CAF) were maintained in complete medium as for HSC-2 cells. Early passage (below passage 10) of the fibroblasts were subjected to analysis.

Reagents. All reagents used in cell culture were purchased from Gibco BRL Co. (Rockville, MD, USA). Cholera toxin, hydrocortisone, insulin, apo-transferrin, T3, and dimethyl sulfoxide (DMSO) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Recombinant human interleukin 1 beta (IL-1β) was purchased from R&D Systems (Minneapolis, MN, USA). IL-1R antagonist was purchased from Cayman (Cayman Chemical, Ann Arbor, MI, USA). GM6001 was purchased from Calbiochem (La Jolla, CA, USA). Oregon Green 488 gelatin were purchased from Molecular Probes (Carlsbad, CA, USA).

Invasion assay. 8 μm pore sized polycarbonate nucleopore filter inserts in a 24-well Transwell chamber (Corning Costar, Cambridge, MA, USA) were coated with Matrigel (30 mg/well; Becton Dickinson, Lincoln Park, NJ, USA) for 4 h. Cells ($5x10^4$ cells) were added into the upper chamber, and complete medium was added to the bottom chamber and keep for 48 h at 37˚C incubator. Invaded cells on the lower surface of the Transwell membrane was fixed with absolute ethanol and noninvasive cells were removed with a cotton swab. Then invaded cells were stained with hematoxylin. Cells from five fields were counted under a microscope. The neutralizing effect of anti-ADAM 9 antibody and anti-Kallikrein 11 antibody on the invasion activity of CM-CAF was determined by incubating the cells with CM-CAF after CM-CAF were treated for 1 h with 1 mg/ml antibody to ADAM 9 (Abcam, Cambridge, MA, USA) and anti-Kallikrein 11 (R&D Systems, Saint Louis, MO, USA).

Immunostaining. The xenograft tumor tissue was used to observe the expression of IL-1β. Tumor xenograft was established by implatation of OSCC cells in athymic nude mouse in our previous study (8). Briefly, OSCC cells were injected into mouse tongue and growth of tumor xenografts was observed for 5 weeks. Tissues were fixed in formalin and processed for paraffin embedding. Deparaffinized tissue were then rehydrated and conducted antigen retrieval via autoclave treatment of the sections in 0.01 M citrate buffer (pH 6.0). After blocking with 10% normal goat serum, the sections were incubated with a primary antibody at a 1:100 dilution in background reducing diluent (Dako, Carpinteria, CA, USA). The sections were rinsed with PBS and incubated with biotinylated anti-mouse/anti-rabbit IgG (H + L) (1:100 dilution in background reducing diluent), followed exposure to horseradish peroxidase streptavidin (1:200 dilution in background reducing diluent). Staining was performed by incubating with 3,3'-diaminobenzidine (DAB) buffer. The sections were counterstained with hematoxylin, followed by dehydration and mounting.

Enzyme-linked immunosorbent assay (ELISA). The cultured medium from NF and CAF were used as conditioned medium (CM). Media were then centrifuged, and the IL-1β level was quantified with the Human IL-1β Quantikine ELISA kit according to the manufacturer's protocols (R&D System Inc., Minneapolis, MN, USA).

ECM degradation. Oregon Green 488 gelatin-coated coverslips were prepared as described previously (9). Cells ($3x10^3$ cells) were plated on coverslips in 12-well plates and cultured for 16 h. Cells were fixed with 4% paraformaldehyde followed by permeabilization with 0.5% Triton X-100/PBS and stained for nuclei with DAPI. Areas of matrix degradation was identified by a loss fluorescence using an EVOS FL monochrome microscope (ThermoFisher Scientific, Waltham, MA, USA).

Protease array. Cells were cultured in 1% medium with or without IL-1β and harvested after 24 h. Harvested medium was centrifuged in 5,000 rpm for 10 min and used as conditioned media (CM) for protease array. The protein concentration of the CM was normalized by dilution with serum-free media. Then CM was incubated for 24 h with the Proteome Profiler Human Protease Array membrane (R&D Systems, Saint Louis, MO, USA). The relative expression levels of the proteases were determined according to the manufacturer's protocol and signal intensities were compared using ImageJ software program.

Statistical analysis. The statistical analysis was conducted using InStat™ statistical software (GraphPad Software, San Diego, CA, USA). The statistical significance of differences between groups was analyzed using a one-way ANOVA with a Tukey's post-hoc test. P-values of <0.05 were considered significant.

Results

IL-1β increases cancer cell invasion. To investigate the effect of IL-1β in cancer invasion, a Matrigel-coated Transwell invasion assay was performed with 20 ng/ml IL-1β for 48 hours. As shown in Fig. 1, recombinant IL-1β treatment incresed

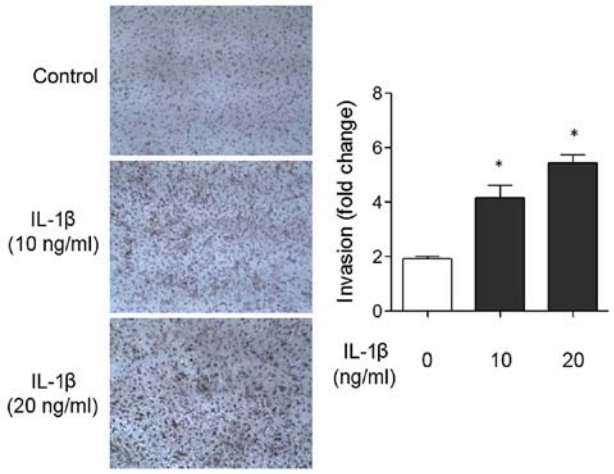

Figure 1. Cancer invasion by IL-1β stimulation. Matrigel-coated transwell invasion was performed with or without recombinant IL-1β protein. An invasion assay was performed for 48 h. Cell invasion was detected by hematoxylin staining and counted. Representative results of the transwell invasion assay are shown. *P<0.01 vs. no IL-1β stimulation. IL, interleukin.

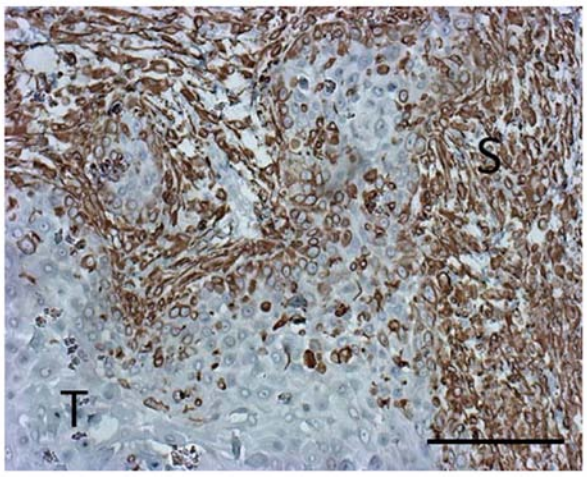

Figure 2. Expression of IL-1β in tumor xenograft. Representative immunohistochemical staining images of IL-1β on xenografted tongue tumor specimen (original magnification, x100). Scale bar indicates 50 μm. IL, interleukin; T, tumor; S, stroma.

invasion 2.75-fold compared to the control. To investigate the expression of IL-1β in the tumor, we used a xenofrafted tongue tumor specimen established in our previous experiments (8). IL-1β expression was located in the cytoplasm of the cancer cells and cancer-associate fibroblasts. Relatively high IL-1β expression was detected in the regional stroma (Fig. 2).

IL-1β from CAFs increases matrix degradation. The level of IL-1β protein was measured in normal gingival fibroblasts (NFs) and cancer-associated fibroblast (CAFs) using a Human IL-1β Quantikine ELISA kit. As shown in Fig. 3A, IL-1β expression was 3.8-fold higher in CAF than in NF. Conditioned medium (CM) from NF and CAF were used for Transwell invasion assay, instead of complete medium. Unlike NF, CAF increased cancer inasion by 2.1-fold (Fig. 3B). The increased invasion activity by the CM of CAF (CM-CAF) was abolished by an IL-1R antagonist. Subsequently, we observed whether the invasion activity of cancer cells increased by CM-CAF increased matrix degradation. Cancer cells were cultured for 16 h on FITC-gelatin-coated coverslips. CM-CAF stimulation increased the degradation activity of FITC-gelatin matrix compared to the control without CM-CAF (Fig. 3C). The increased matrix degradation by CM-CAF stimulation was abolished by IL-1R antagonist treatment. These results indicate that CM-CAF increased proteolytic cancer invasion in an IL-1R-dependent manner.

IL-1β increases the protease release. In order to explore the proteases that increased cancer invasion by CM-CAF, the cells were stimulated with CM-CAF and culture medium was analyzed using the Proteome Profiler Human Protease Array. Compared to control, the secretion of ADAM 9 and Kallikrein 11 was significantly increased by CM-CAF treatment. ADAM 9 and Kallikrein 11 were increased 4.21- and 2.48-fold, respectively (P<.01) (Fig. 4). The increased in Transwell invasion by CM-CAF was partially abrogated by the neutralization of ADAM 9 or Kallikrein 11 (Fig. 5).

Discussion

Protease secretion by cancer cells is a pivotal process in cancer invasion and metastasis. Invasive cancer cells form a protrusive cellular structure, termed invadopodia, for effective cancer invasion, leading to the focal secretion of proteases into the nearby extracellular matrix (ECM) (10,11). Growth factors, such as colony stimulating factor-1 (CSF-1), TGF-β, VEGF, PDGF, EGF, heparin-binding EGF (HB-EGF), hepatocyte growth factor/scatter factor (HGF), and stromal cell-derived factor 1α (SDF1α) stimulate invadopodia formation (10). MMPs, ADAM (a disintegrin and metalloproteinase), sheddases, cysteine cathepsin proteases, and serine proteases are localized at invadopodia (12,13). TGF-β and EGF are synthesized in active proforms that are processed by MMPs and another protease into active and soluble ligands, suggesting a possible role for protease in invadopodia formation (10,14).

In the present study, IL-1β concentration was measured in normal fibroblast (NF) and cancer-associated fibroblast (CAF), and higher IL-1β levels were observed in the CAF compared to the NF. Recombinant IL-1β significantly increased cancer cell invasion on Matrigel-coated Transwell chamber. Further, we investigated whether stromal fibroblasts could control cancer invasion. Conditioned media (CM) from NF and CAF were prepared and used as cultured media in Transwell chamber for invasion assay. Unlike CM from NF, CM from CAF (CM-CAF) increased cancer cell invasion by increasing protease release. The invasive capacity provided by CM-CAF was suppressed by an IL-1 receptor (IL-1R) antagonist. These results indicate that the level of IL-1β in CM-CAF was high enough to stimulate cancer invasion activity and that the increased invasion activity of the cancer cells resulting from CM-CAF treatment was sustained by an IL-1R-dependent pathway. IL-1β is a pro-inflammatory cytokine and its expression in primary tumors has been identified as a potential biomarker in cancer patients at increased risk for developing bone metastasis (15). IL-1 has been suggested as a critical molecule for tumor invasiveness and angiogenesis (16). Local tumors and lung metastases of B16 melanoma cells were abolished in IL-1

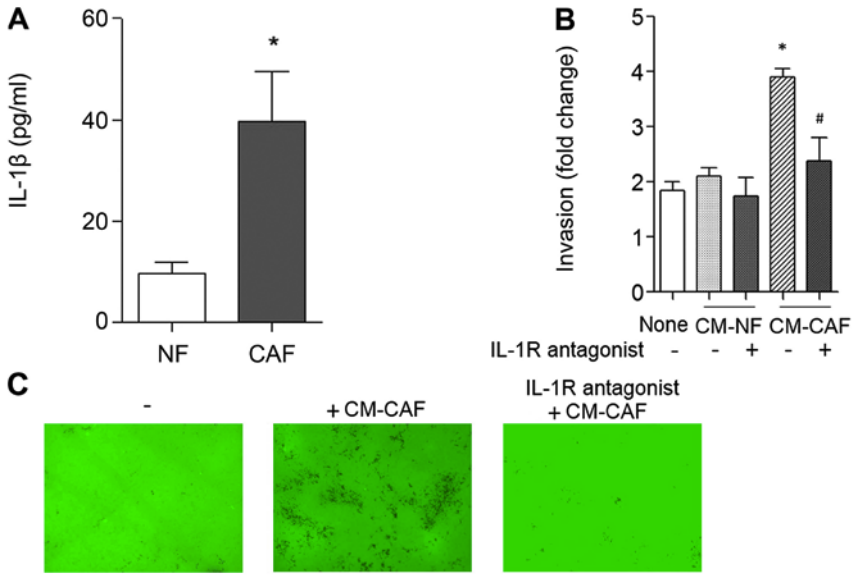

Figure 3. Effect of CAF on cancer invasion activity. (A) The levels of IL-1β in NF and CAF were measured using a Human IL-1β Quantikine ELISA kit. *P<0.01 vs. NF. (B) Effect of CM from NF and CAF on cancer invasion was estimated with or without IL-1 receptor (IL-1R) antagonist. *P<0.01 vs. none, #P<0.01 vs. without IL-1R antagonist in CAF. (C) Extracellular matrix degradation activity of cancer cells by CM from CAF (CM-CAF). Cells were cultured on Oregon Green 488 gelatin-coated coverslips and areas of matrix degradation were identified by a loss fluorescence using an EVOS FL monochrome microscope (original magnification, x100). IL-1(R) antagonist; IL-1 receptor antagonist. CAF, cancer-associated fibroblast; IL, interleukin; NF, normal fibroblast; CM, conditioned media.

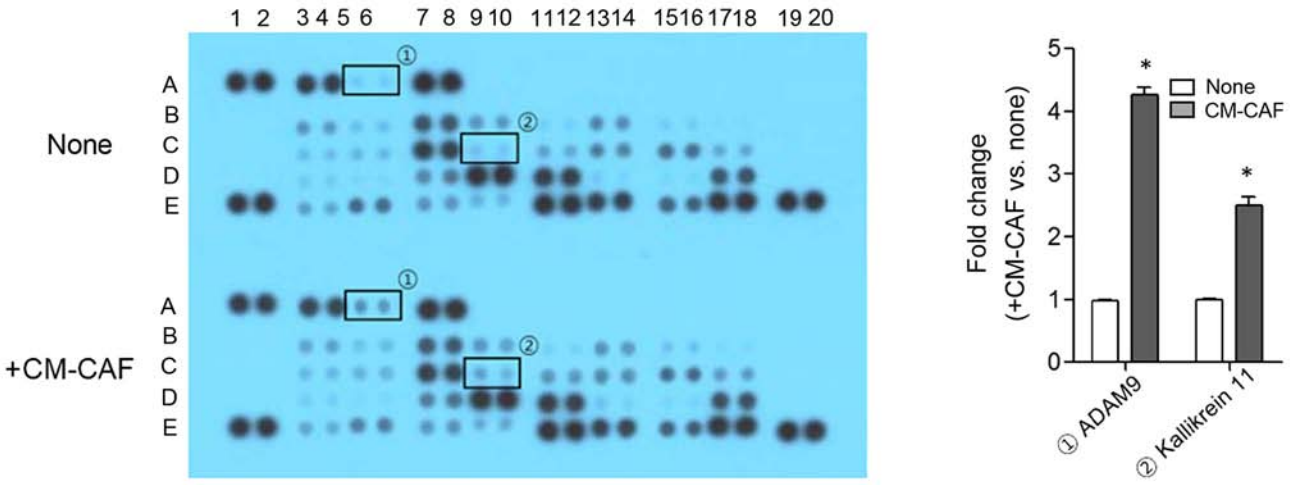

Figure 4. Effect of CM from CAF (CM-CAF) stimulation on proteases release from oral squamous cell carcinoma cells. CM-CAF was incubated with Protease Array membrane and relative signal intensities were determined using ImageJ software program. Proteases with large signal changes are indicated by circled numbers. *P<0.001 vs. none. CM, conditioned media; CAF, cancer-associated fibroblast.

knockout (KO) mice. IL-1β secreted by OSCC cells reciprocally stimulated secretion of transforming growth factor beta 1 (TGF-β1) from stromal fibroblasts, thereby leading to cancer invasion by increasing podoplanin (PDPN) expression, one of the components of invadopodia (17). These results indicate that cancer invasion is regulated by reciprocal cross-talk between cancer and the regional microenvironment. Thus, the influence of the tumor microenvironment is important in elucidating the mechanism of cancer invasion. According to a recent report, cancer-associated fibroblasts also formed invadopodia, thereby promoting invasion activity of pancreatic cancer cells (18).

The stroma consists of the basement membrane, fibroblasts, extracellular matrix, immune cells, and vascular system and plays a structural and connective role in tissues. Normal stroma maintains homeostasis by inhibiting inflammation and neoplasia through immune systems functions. However, in the tumor environment, it plays a role in promoting cancer growth and malignant tumors (19). Cancer-associated fibroblasts (CAFs) are abundant cells within the tumor microenvironment and promote tumorigenic features by initiating remodeling of the extracellular matrix (ECM) and secreting cytokines. CAFs are continuously activated and lack the ablility to revert into a normal phenotype or undergo apoptosis, resulting in a constant number of CAFs. (19). Consequently, the reciprocal cross-talk between stromal tissue and tumor plays a pivotal role in cancer growth and progression (20). We previously demonstrated that CAFs promoted tumor growth in athymic nude mice (3). Tumor growth was significantly increased

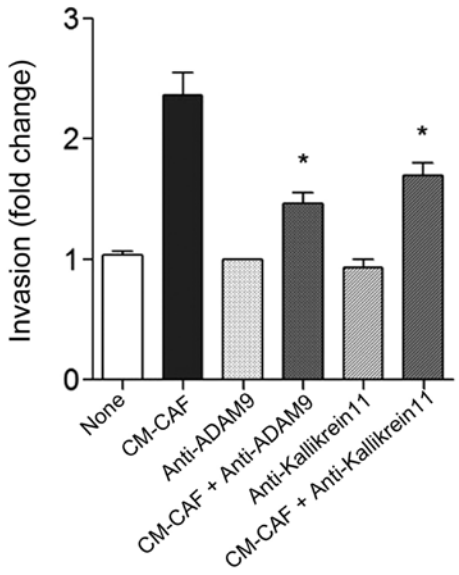

Figure 5. Effect of neutralization of ADAM 9 or Kallikrein 11 on Transwell invasion activity by CM-CAF. CM-CAF were treated with 1 mg/ml antibody to ADAM 9 or anti-Kallikrein 11 for 1 h and were added to the lower chamber of Transwell plates. After incubation, the invaded cells were stained and counted. Results represent the mean ± standard error of three independent experiments. *P<0.01 vs. CM-CAF alone. CM, conditioned media; CAF, cancer-associated fibroblast.

in xenograft mixtures of CAF and cancer cell compared to xenografts of cancer cell only in a 5-weeks experiment. This result indicates that the existence of molecular cross-talk between cancer cells and surrounding stroma is important for enhancing tumor growth. Thus, targeting of CAFs is important for understanding cancer invasion and finding keys to suppress cancer progression. However, there is little research on the role of IL-1β in CAF-mediated stimulation of cancer invasion. In this study, we demonstrated that CM-CAF contained significant amounts of IL-1β and induced the secretion of proteases from OSCC cells.

In the present study, CM-CAF was shown to stimulate the enhanced secretion of ADAM 9 (MDC9, meltrin β) and Kallikrein 11 (KLK11, TLSP, PRSS20) from OSCC cells. The increased Transwell invasion by CM-CAF was abrogated by neutralization of ADAM 9 or Kallikrein 11. ADAM 9 is known as metalloprotease disintegrin cysteine-rich protein 9 or meltrin β. Overexpression of ADAM 9 has been identified in a variety of cancer types, including breast cancer, renal cancer, prostate cancer, skin cancer, uterine cervical cancer, hepatocellular carcinoma, non-small cell lung cancer, colon cancer, gastric cancer, esophageal cancer, and head and neck squamous cell carcinoma (HNSCC) (21). ADAM 9 is induced by oxidative stress, such as intracellular reactive oxygen species (ROS) and/or hydrogen peroxide, thereby supporting prostate cancer cell survival and progression (22). Enhanced ADAM 9 expression has been reported in oral squamous cell carcinoma (OSCC) compared to normal oral tissue. Moreover, a high degree of ADAM 9 expression has been observed in well-differentiated OSCC (21). Kallikrein 11 (KLK11) is a secreted type of serine protease, highly expressed in many tissues including brain, skin, salivary gland, stomach, prostate, and intestine (23). KLK11 expression holds prognostic significance in prostate cancer (24). *In vivo* studies have demonstrated that overexpression of KLK11 led to tumor progression and metastasis of prostate cancer. Overexpression of KLK11 in ER(+) breast cancer cells led to breast cancer progression by increasing the bioavailability of IGFs via degradation of insulin-like growth factor (IGF) binding protein 3 (IGFBP-3) (25). Previously, KLK11 has been proposed as a diagnostic biomarker of prostate and ovarian carcinoma (26). IL-1β increased the expression of an ADAM 9 in hepatocellular carcinoma (HCC) cell to escape from the host immune surveillance (27). The relation between IL-1β and KLK11 has not been reported. Neutralization of IL-1 receptor (IL-R) will be a useful tool in elucidating the mechanisms involved in IL-1β-induced ADAM 9 or KLK11 production. The role of ADAM 9 and KLK11 induced by CM-CAF in OSCC cell invasion also require further investigations. Identification of the cellular mechanism of protease controlled by stromal factor IL-1β would be of immense significance.

In conclusion, CAFs were observed to increase cancer invasion in an IL-1R-dependent manner. The release of ADAM 9 and Kallikrein 11 from OSCC cells was also stimulated by CAFs. A study on the molecular mechanism of CAF-induced proteases secretion from cancer cells is needed to further investigate cancer invasion. Targeting the cancer microenvironment is important for cancer control.

Acknowledgements

Not applicable.

Funding

The current research was supported by Eulji University in 2019 and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (grant no. 2018R1D1A1B07042035).

Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

XZ and YSH performed the experiments, analyzed and interpreted data and wrote the manuscript.

Ethics approval and consent to participate

Approval was received from the Animal Ethics Committee of Eulji University (permit no. EUIACUC17-18).

Patients consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Wang M, Zhao J, Zhang L, Wei F, Lian Y, Wu Y, Gong Z, Zhang S, Zhou J, Cao K, *et al*: Role of tumor microenvironment in tumorigenesis. J Cancer 8: 761-773, 2017.
2. Chen F, Zhuang X, Lin L, Yu P, Wang Y, Shi Y, Hu G and Sun Y: New horizons in tumor microenvironment biology: Challenges and opportunities. BMC Med 13: 45, 2015.
3. Hwang YS, Park KK and Chung WY: Stromal transforming growth factor-beta 1 is crucial for reinforcing the invasive potential of low invasive cancer. Arch Oral Biol 59: 687-694, 2014.
4. Yuan Y, Jiang YC, Sun CK and Chen QM: Role of the tumor microenvironment in tumor progression and the clinical applications (review). Oncol Rep 35: 2499-2515, 2016.
5. Zi F, He J, He D, Li Y, Yang L and Cai Z: Fibroblast activation protein α in tumor microenvironment: Recent progression and implications (review). Mol Med Rep 11: 3203-3211, 2015.
6. Raman D, Baugher PJ, Thu YM and Richmond A: Role of chemokines in tumor growth. Cancer Lett 256: 137-165, 2007.
7. Hassona Y, Cirillo N, Heesom K, Parkinson EK and Prime SS: Senescent cancer-associated fibroblasts secrete active MMP-2 that promotes keratinocyte dis-cohesion and invasion. Br J Cancer 111: 1230-1237, 2014.
8. Zhang X, Cho IH, Park JH, Lee MK and Hwang YS: Fascin is involved in cancer cell invasion and is regulated by stromal factors. Oncol Rep 41: 465-474, 2019.
9. Hwang YS, Lee J, Zhang X and Lindholm PF: Lysophosphatidic acid activates the RhoA and NF-κB through Akt/IκBα signaling and promotes prostate cancer invasion and progression by enhancing functional invadopodia formation. Tumour Biol 37: 6775-6785, 2016.
10. Hoshino D, Branch KM and Weaver AM: Signaling inputs to invadopodia and podosomes. J Cell Sci 126: 2979-2989, 2013.
11. Lee D, Yu EJ, Ham IH and Hur H: Clinicopathological implication of insulin-like growth factor-II mRNA-binding protein 3 (IMP3) expression in gastric cancer. Anticancer Res 37: 135-142, 2017.
12. Murphy DA and Courtneidge SA: The 'ins' and 'outs' of podosomes and invadopodia: Characteristics, formation and function. Nat Rev Mol Cell Biol 12: 413-426, 2011.
13. Klemke RL: Trespassing cancer cells: 'Fingerprinting' invasive protrusions reveals metastatic culprits. Curr Opin Cell Biol 24: 662-669, 2012.
14. Kessenbrock K, Plaks V and Werb Z: Matrix metalloproteinases: Regulators of the tumor microenvironment. Cell 141: 52-67, 2010.
15. Tulotta C and Ottewell P: The role of IL-1B in breast cancer bone metastasis. Endocr Relat Cancer 25: R421-R434, 2018.
16. Voronov E, Shouval DS, Krelin Y, Cagnano E, Benharroch D, Iwakura Y, Dinarello CA and Apte RN: IL-1 is required for tumor invasiveness and angiogenesis. Proc Natl Acad Sci USA 100: 2645-2650, 2003.
17. Hwang YS, Xianglan Z, Park KK and Chung WY: Functional invadopodia formation through stabilization of the PDPN transcript by IMP-3 and cancer-stromal crosstalk for PDPN expression. Carcinogenesis 33: 2135-2146, 2012.
18. Goicoechea SM, García-Mata R, Staub J, Valdivia A, Sharek L, McCulloch CG, Hwang RF, Urrutia R, Yeh JJ, Kim HJ and Otey CA: Palladin promotes invasion of pancreatic cancer cells by enhancing invadopodia formation in cancer-associated fibroblasts. Oncogene 33: 1265-1273, 2014.
19. De Veirman K, Rao L, De Bruyne E, Menu E, Van Valckenborgh E, Van Riet I, Frassanito MA, Di Marzo L, Vacca A and Vanderkerken K: Cancer associated fibroblasts and tumor growth: Focus on multiple myeloma. Cancers (Basel) 6: 1363-1381, 2014.
20. Wang FT, Sun W, Zhang JT and Fan YZ: Cancer-associated fibroblast regulation of tumor neo-angiogenesis as a therapeutic target in cancer. Oncol Lett 17: 3055-3065, 2019.
21. Tanasubsinn P, Aung WPP, Pata S, Laopajon W, Makeudom A, Sastraruji T, Kasinrerk W and Krisanaprakornkit S: Overexpression of ADAM9 in oral squamous cell carcinoma. Oncol Lett 15: 495-502, 2018.
22. Sung SY, Kubo H, Shigemura K, Arnold RS, Logani S, Wang R, Konaka H, Nakagawa M, Mousses S, Amin M, *et al*: Oxidative stress induces ADAM9 protein expression in human prostate cancer cells. Cancer Res 66: 9519-9526, 2006.
23. Yousef GM, Scorilas A and Diamandis EP: Genomic organization, mapping, tissue expression and hormonal regulation of trypsin-like serine protease (TLSP PRSS20), a new member of the human kallikrein gene family. Genomics 63: 88-96, 2000.
24. Stavropoulou P, Gregorakis AK, Plebani M and Scorilas A: Expression analysis and prognostic significance of human kallikrein 11 in prostate cancer. Clin Chim Acta 357: 190-195, 2005.
25. Sano A, Sangai T, Maeda H, Nakamura M, Hasebe T and Ochiai A: Kallikrein 11 expressed in human breast cancer cells releases insulin-like growth factor through degradation of IGFBP-3. Int J Oncol 30: 1493-1498, 2007.
26. Diamandis EP, Okui A, Mitsui S, Luo LY, Soosaipillai A, Grass L, Nakamura T, Howarth DJ and Yamaguchi N: Human kallikrein 11: A new biomarker of prostate and ovarian carcinoma. Cancer Res 62: 295-300, 2002.
27. Kohga K, Tatsumi T, Tsunematsu H, Aono S, Shimizu S, Kodama T, Hikita H, Yamamoto M, Oze T, Aketa H, *et al*: Interleukin-1β enhances the production of soluble MICA in human hepatocellular carcinoma. Cancer Immunol Immunother 61: 1425-1432, 2012.