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Irradiated fibroblasts promote epithelial–mesenchymal transition and HDGF expression of esophageal squamous cell carcinoma

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ABSTRACT

Recent evidence suggested that nonirradiated cancer-associated fibroblasts (CAFs) promoted aggressive phenotypes of cancer cells through epithelial–mesenchymal transition (EMT). *Hepatoma-derived growth factor (HDGF)* is a radiosensitive gene of esophageal squamous cell carcinoma (ESCC). This study aimed to investigate the effect of irradiated fibroblasts on EMT and HDGF expression of ESCC. Our study demonstrated that coculture with nonirradiated fibroblasts significantly increased the invasive ability of ESCC cells and the increased invasiveness was further accelerated when they were cocultured with irradiated fibroblasts. Scattering of ESCC cells was also accelerated by the supernatant from irradiated fibroblasts. Exposure of ESCC cells to supernatant from irradiated fibroblasts resulted in decreased E-cadherin, increased vimentin *in vitro* and β-catenin was demonstrated to localize to the nucleus in tumor cells with irradiated fibroblasts *in vivo* models. The expression of HDGF and β-catenin were increased in both fibroblasts and ESCC cells of irradiated group *in vitro* and *in vivo* models. Interestingly, the tumor cells adjoining the stromal fibroblasts displayed strong nuclear HDGF immunoreactivity, which suggested the occurrence of a paracrine effect of fibroblasts on HDGF expression. These data suggested that irradiated fibroblasts promoted invasion, growth, EMT and HDGF expression of ESCC.

1. Introduction

Radiotherapy is a primary treatment of ESCC and the 5-year survival rate of it has still remained to be less than 20% [1]. Recently, several studies suggested that the presence of activated CAFs was associated with an unfavorable outcome [2], local recurrence after definitive chemoradiation for ESCC [3]. The classic paradigm of radiation as a cancer initiator is broadened by studies showing that radiation also influences cancer processes through promoting effects on the stromal microenvironment that are mediated by radiation [4]. These data suggest that targeting stromal fibroblasts may be an attractive approach for ESCC therapy strategies and the precisely mechanism still need to be explored.

Recently, HDGF, an acidic polypeptide with mitogenic activity for fibroblasts and a negative prognostic marker for survival in cancer progression [5], has attracted much attention and been proved to be involved in the regulation of a myriad of cancer cell activities during cancer transformation, apoptosis, angiogenesis and metastasis. HDGF played an important role in radiosensitivity and correlated with tumor recurrence of ESCC [6,7]. CAFs have been linked to a number of prometastatic capabilities, including induction of EMT [8], which endows cells with migratory and invasive properties and induces stem cell properties [9]. Subsequently, interactions between cancer cells and surrounding stromal fibroblasts have been suggested to play a critical role in tumor invasion and metastasis through EMT [10–14]. Previous reports have shown that γ-ray irradiation to fibroblasts are involved in carcinogenesis and invasive growth of cancer cells that are not exposed to irradiation [15–20]. However, to our knowledge, it's absent whether X-ray irradiation to stromal fibroblasts could facilitate ESCC invasion and metastasis through EMT.

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In the present study, we used X-ray source to irradiate fibroblasts and then investigated the proactive influence of irradiated CAFs on their ESCC cells counterparts and further identified the role of the HDGF and EMT program in this pathological process. The increasing knowledge of the relationship between fibroblasts and response to irradiation could help developing innovative strategies for potentially improve antitumor effect of radiotherapy.

2. Materials and methods

2.1. Culture of ESCC cell lines and antibodies

Two established human ESCC cell lines (Eca109 and Eca9706) were used for this study. Both of the two cell lines were cultured in DMEM (Hyclone) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin G and streptomycin in a 37 °C incubator with humidified atmosphere and 5% CO_2.

Antibodies used were anti-HDGF mouse monoclonal antibody, anti-a-smooth muscle actin(a-SMA) rabbit polyclonal antibody, anti-β-catenin rabbit polyclonal antibody (Proteintech Group, Inc., Chicago, USA), anti-fibronectin goat polyclonal antibody, anti-β-actin mouse monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-vimentin mouse monoclonal antibody (Boster Biologicals, China) and anti-E-cadherin monoclonal antibody (Novus Biologicals, USA).

2.2. Isolation and culture of fibroblasts

Primary ESCC tissues and normal fibroblasts (NFs) derived from primary prepuce were collected at the time of surgical resection at the Qilu Hospital of Shandong University (QLHSDU; Jinan, China). The two cancer patients (both male, >50 years old, poorly differentiated ESCC with lymphatic vessel invasion and lymphatic metastasis) have not received adjuvant therapy before surgery. Specimens used in this study were approved by the Ethics Committee of QLHSDU and written consent was obtained from participants.

The tissue specimens were divided into two parts for histopathological diagnosis and isolation of fibroblasts. CAFs were isolated from primary ESCC as described previously [21]. The fresh tissues were sliced and digested with 0.1% collagenase type IV (Sigma) at 37 °C for 30 min. Then the cells were collected and cultured in 75 cm^2 culture flask in 12 ml DMEM (Hyclone, USA) supplemented with 20% FBS, 100 U/ml penicillin G and streptomycin at 37 °C with humidified 90% air and 10% CO_2 until cells grew into a confluent monolayer. The method of isolating NFs was similar to CAFs.

2.3. Immunofluorescence cell staining

Cells were grown on gelatin-coated coverslips for 24 h and then fixed in 4% paraformaldehyde at room temperature for 20 min. Cells were incubated with 5% BSA (Sigma) solution at 37 °C for 30 min to block nonspecific interactions and stained with primary antibodies targeting fibronectin at 4 °C overnight. After several washes in PBS, cells were incubated with optimal concentrations of FITC-labelled rabbit anti-goat IgG (H + L) secondary antibody (ZhongshanGoldenbridge Biological Technology, Beijing, China) at room temperature for 1 h. Anti-fade DAPI solution was added and images were obtained (Olympus BX53, Tokyo, Japan).

2.4. Radiation

Cells were irradiated with different doses at room temperature using X-rays source (6 MV; Varian 23 EX; Varian, Ltd., USA) with a delivering rate of 400 cGy/min. Fibroblasts were seeded at a density of 1.5×10^6 cells in 10-cm dishes and irradiated. Nonirradiated controls were removed from the cell culture incubator for the same period as irradiated samples. To prepare conditioned medium of cultured CAFs (CAF-CM) for ESCC cell treatments, stromal fibroblasts isolated from two tissue specimens were mixed and cultured for 48 h, then collected and centrifuged for 30 min at 1500 r.p.m. to remove cell debris.

2.5. Invasion assay

Invasion of ESCC cells was measured by the number of cells invading through matrigel-coated transwell inserts (BD Bioscience). Briefly, transwell inserts (Corning) with 8-μm pores were coated with matrigel (matrigel/DMEM = 8:1; 60 μl/well; BD Bioscience). Fibroblasts were initially seeded on a 24-well plate at a density of $2–5 \times 10^4$/well and cultured in DMEM supplemented with 10% FBS for 24 h. These fibroblasts were then irradiated at 4 or 8 Gy, and the medium was immediately replaced with 600 μl of fresh medium. Cancer cells were seeded at a density of 1×10^5/well in the upper chambers with 250 μl of DMEM supplemented without FBS. After 24 h of incubation, cells that had invaded to lower surface of the matrigel-coated membrane were fixed with methanol, stained with 0.1% crystal violet, and counted in five randomly selected fields under a light microscope (Olympus BX51, Tokyo, Japan).

2.6. Scattering assay

ESCC cells were plated at a density of 1500 cells/well in DMEM containing 10% FBS into 6-well plates. After cultivation for 7 days, cells were stimulated by CM of irradiated and nonirradiated fibroblasts. Scattering of cells was evaluated under a phase-contrast microscope (Olympus BX51) at 24 h after stimulation.

2.7. Western blotting

ESCC cells were cultured with supernatant of irradiated or nonirradiated fibroblasts for the indicated hours. Proteins expression of fibroblasts was also detected using western blotting. Protein extraction and western blotting were conducted as described previously [22]. Specific antigen–antibody interactions were detected with enhanced chemiluminescence (Image Quant LAS 4000mini).

2.8. In vivo model

ESCC cells (Eca-109, 1.2×10^6; Eca-9706, 1.5×10^6) and 0.5×10^6 4Gy- or non-irradiated fibroblasts were mixed well and implanted subcutaneously into the right flank of 4–5 week-old female nude mice (BALB/c nu/nu; Beijing HFK Bio-Technology.co., LTD, China). Six mice were used in each group. Tumor size was measured every 3 days with the use of a caliper. The tumor volume was calculated according to the formula volume = (length × $width^2$)/2. Tumors were excised and weighted after 24 days and then fixed in 4% paraformaldehyde for 24 h and embedded in paraffin according to standard histological procedures. All animal studies were approved by the Ethics Committee of QLHSDU and conducted in compliance with UKCCCR guidelines.

2.9. Immunohistochemistry

Tissue sections were deparaffinized in xylene and rehydrated in graded alcohols and distilled water. Slides were processed for antigen retrieval by a standard microwave heating technique. Specimens were incubated with anti-HDGF or anti-β-catenin antibodies

(1:100 dilution). The immunoreactions were visualized with diaminobenzidine as a chromogen and counterstained with hematoxylin. The slides were washed in tap water, dehydrated in alcohol, and mounted (Olympus BX53). Stained sections were evaluated in a blinded manner without prior knowledge of the clinical information. Nuclear and cytoplasmic HDGF expression was independently evaluated. Tumor cells showing nuclear staining that was equal to or stronger than the staining intensity in endothelial cells were considered nuclear positive. The same process was followed for cytoplasmic staining. Cases with >90% of tumor cells showing nuclear positive staining and >90% of tumor cells showing cytoplasmic positive staining were categorized as level 1 HDGF staining, and the remaining cases as level 0 HDGF staining [7].

2.10. Statistical analysis

All data were shown as mean ± SD. Comparisons between three or more groups were done by one-way ANOVA and by the Student's t test for comparison between two groups. Differences with P value <0.05 were considered statistically significant.

3. Results

3.1. Isolation, characterization and purity of fibroblasts

To characterize stromal fibroblasts in the microenvironment of ESCC, we isolated and cultured stromal fibroblasts from primary ESCC tissues. In the primary ESCC tissues, we observed that stromal fibroblasts surrounding cancer cells highly expressed activated fibroblast marker a-SMA, present characteristics of CAFs (Fig. 1A). The stromal cells separated from primary ESCC tissues were heterogeneous mixture of a variety of cells in the primary culture, with fibroblasts being the main component. After culturing for 30 min at 37 °C, unadherent cells (mainly tumor cells) were removed to obtain pure fibroblasts because the adhesion time needed for fibroblasts is much shorter (~20–30 min) than that for tumor cells (usually >1 h) [21]. After 2–3 passages, uniform fibroblasts started to grow (Fig. 1B). To confirm that CAFs were pure fibroblasts without other cells contamination, the fibroblast marker fibronectin was used to distinguish fibroblasts from ESCC cells (Fig. 1C) [21]. The primary cultured stromal fibroblasts were detected by western blotting and expressed a-SMA highly but did not express E-cadherin, presenting characteristics of CAFs. These results indicated that the isolated and cultured fibroblasts *in vitro* from ESCC tissues maintained the features of CAFs. All the fibroblasts used in the experiments were at less than 10 passages and showed spindle-like morphology (Fig. 1B). E-cadherin was not expressed in any fibroblasts after 3 passages, which indicated that the fibroblasts at low passages cultured *in vitro* retained the features of fibroblasts.

3.2. The supernatant from irradiated fibroblasts promotes scattering of ESCC cells

Because the scattering of epithelial colonies possesses characteristics of EMT, such as the loss of epithelial cell–cell junctions and the acquisition of a motile mesenchymal cell phenotype, the scatter

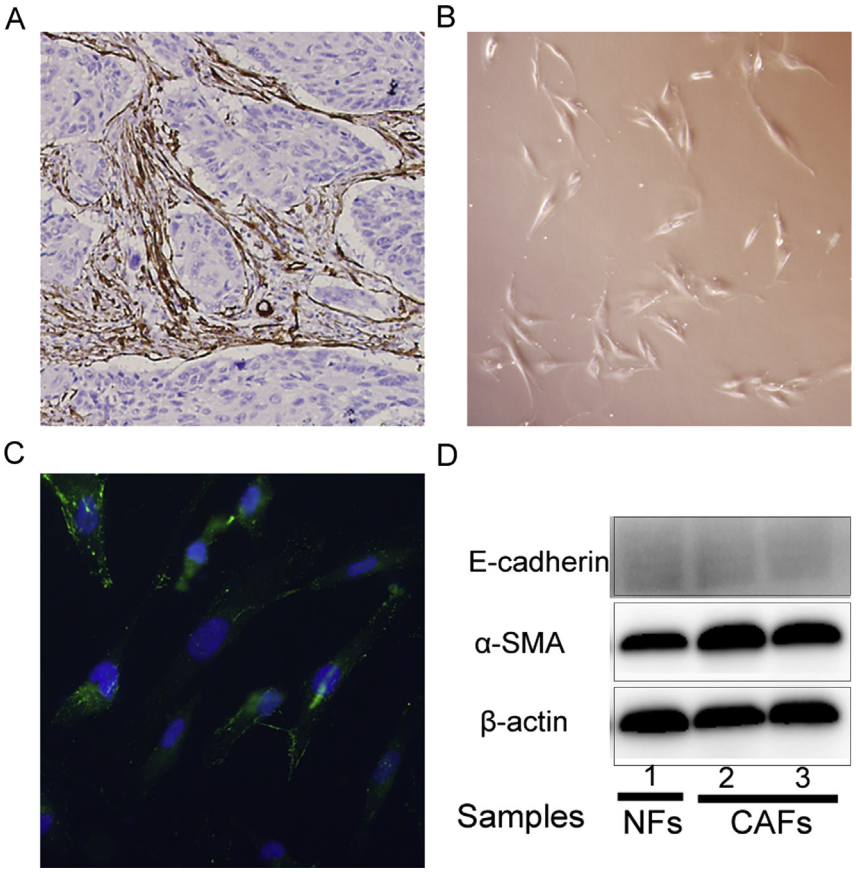

Fig. 1. Stromal fibroblasts isolated from patients exhibit characteristics of CAFs. (A) Stromal fibroblasts surrounding cancer cells highly expressed activated fibroblast marker a-SMA, present characteristics of CAFs (original magnification, 100 ×). (B) Representative cell morphology of CAFs. (C) CAFs expressed fibroblast marker fibronectin (original magnification, 200 ×). (D) All the primary cultured stromal fibroblasts expressed a-SMA highly but did not express E-cadherin, presenting characteristics of CAFs.

assay has been used for studying EMT and for detecting factors able to induce migratory behavior of cells [23]. We then examined the effect of the supernatant derived from irradiated fibroblasts on cell scattering. Exposure to the supernatant from irradiated fibroblasts accelerated the scattering of ESCC cells in a dose-dependent manner (Fig. 2A).

3.3. Irradiated fibroblasts enhanced invasiveness of ESCC cells

Using *in vitro* invasion assay, we examined the invasiveness of ESCC cells (Eca-109 and Eca-9706) cocultured with non-, 4Gy- and 8Gy-irradiated fibroblasts (NFs and CAFs). Only a small number of ESCC cells invaded through matrigel when they were cultured alone, whereas coculture with fibroblasts increased the invasive ability of ESCC cells, which is consistent with previous data in other cancer cells [20,24]. Surprisingly, coculture with fibroblasts after irradiation further enhanced the invasiveness of ESCC cells in a dose-dependent manner. Moreover, coculture with irradiated CAFs (at 4 and 8 Gy) compared with NFs showed significantly larger number of invading cells compared with coculture with nonirradiated counterparts in all two ESCC cell lines tested (Fig. 2B–D).

3.4. Irradiated fibroblasts promote EMT of ESCC cells in vitro and in vivo

To determine whether the effect of irradiated fibroblasts on cell migration and invasion was associated with EMT, expressions of E-cadherin (epithelial marker), vimentin (mesenchymal marker) and β-catenin were compared between irradiated fibroblasts-CM and control-CM cultured ESCC cells at the 24hr time point by western blotting (Fig. 3A and B).The results showed that the expression of all the tested E-cadherin was significantly downregulated, whereas vimentin was slightly upregulated and highly expression in former irradiated groups in a dose-dependent manner. The expression of all the tested β-catenin was upregulated in ESCC cells with irradiated fibroblasts *in vitro* and *in vivo*. Remarkably, enhanced β-catenin was demonstrated to localize to the nucleus in tumors with irradiated fibroblasts *in vivo* (Fig. 3C).

3.5. Irradiation enhanced HDGF expression in both ESCC cells and fibroblasts in vitro and in vivo

Irradiated fibroblasts promoted tumor growth of ESCC cells, especially in Eca-9706 groups (Fig. 4A). The expression of all the tested HDGF was upregulated in irradiated fibroblasts and homologous ESCC cells in a dose-dependent manner (Fig. 4B and C). Because 4Gy-irradiated NFs CM cultured ESCC cells represented most enhanced invasiveness and showed significantly larger variation of proteins expression compared with non-irradiated controls. Thus, ESCC cells admixed with either 4Gy- or non-irradated NFs were inoculated subcutaneously into the right flank of 4–5 week-old female nude mice. As shown in Fig. 4D, clearly enhanced expression of HDGF was found in tumors with irradiated NFs as compared with controls. Moreover, seven (58.3%, Eca-109, 4/6; Eca-9706, 3/6) tumor tissues of mice of irradiated group showed HDGF level 1 staining, while one (8.3%, Eca-109, 1/6; Eca-9706, 0/6) of nonirradiated group showed HDGF level 1 staining. The tumor cells adjoining the stromal fibroblasts displayed strong HDGF immunoreactivity, whereas those tumor cells residing in the central part of tumor region expressed a relatively low level of HDGF, which

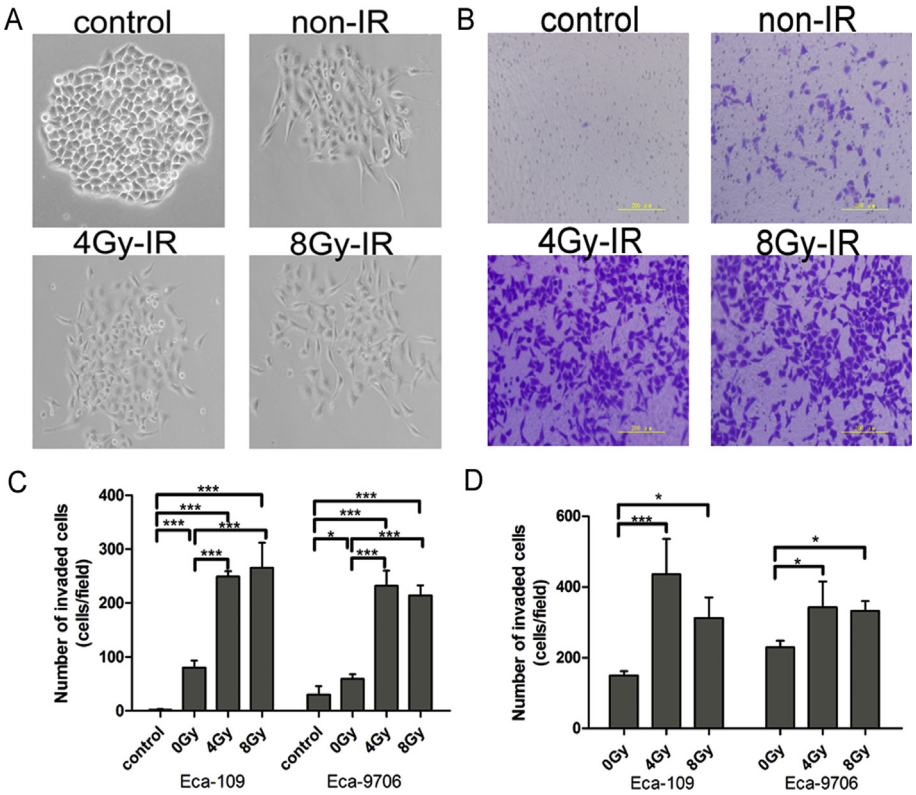

Fig. 2. Irradiated fibroblasts enhanced invasiveness and scattering of ESCC cells. (A) Scattering of Eca-109 before and after exposure to the supernatant from nonirradiated (0 Gy) or irradiated CAFs at 4 or 8 Gy. (B) Photomicrographs of *in vitro* invasion assay in Eca-109 cocultured with non- 4Gy- or 8Gy-irradiated CAFs (Scale bar = 200 μm). Invasive potential of two ESCC cell lines (Eca-109 and Eca-9706) cocultured with irradiated CAFs (C) and NFs (D). Each value represents the mean ± SD (n = 4) of triplicate measurements. *P < 0.01, **P < 0.05, ***P < 0.001.

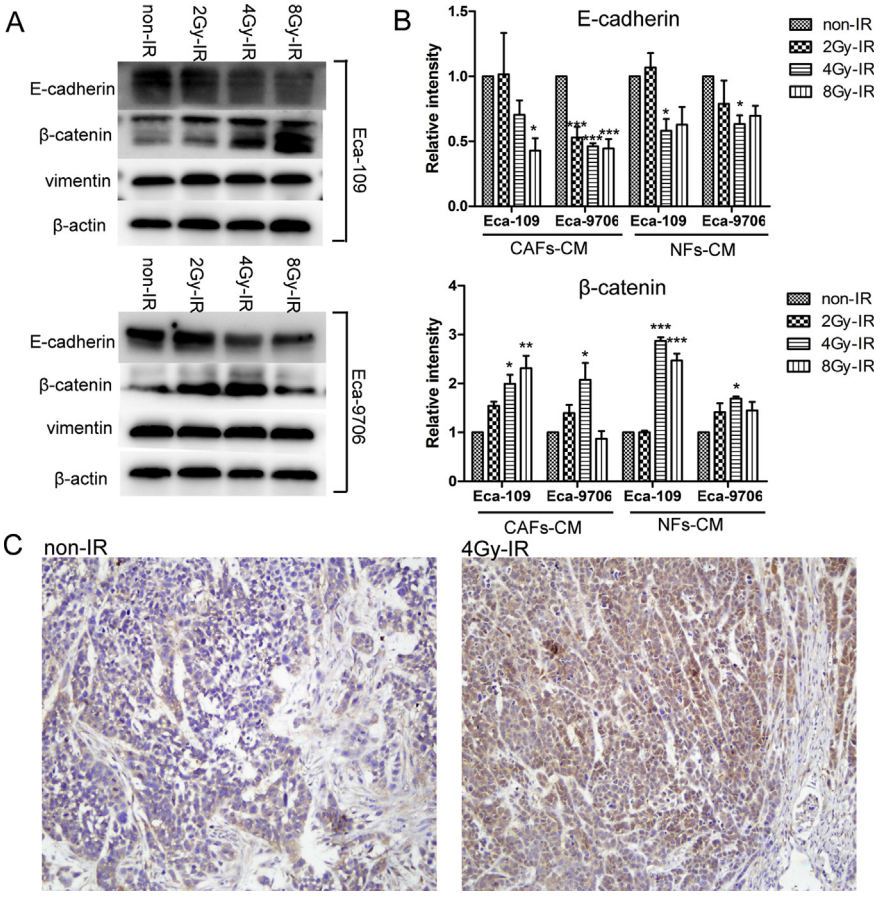

Fig. 3. Irradiated fibroblasts promote EMT of ESCC cells. (A) Exposure of ESCC cells to supernatant from irradiated CAFs resulted in decreased E-cadherin, increased β-catenin *in vitro*. (B) Immunoblot analysis of E-cadherin and β-catenin expression in ESCC cells at 24 h after exposure to the conditioned medium (CM) from either nonirradiated or irradiated CAFs or NFs. All the value in *bar graph* represents a band intensity of E-cadherin and β-catenin (means ± SD of three independent experiments) relative to the intensity at nonirradiated control after normalization by the corresponding β-actin expression. *P < 0.05, **P < 0.01, ***P < 0.001. (C) β-catenin was demonstrated to localize to the nucleus in tumor cells with irradiated fibroblasts *in vivo* models.

suggested the occurrence of a paracrine effect of NFs on HDGF expression. Taken together, the data showed that irradiation enhanced HDGF expression in both ESCC cells and fibroblasts *in vitro* and *in vivo*.

4. Discussion

Isolation of stromal fibroblasts from poorly differentiated ESCC tissues followed by low-passaged cultures *in vitro* is the main approach to investigate the biological characteristics of nonirradiated or irradiated CAFs and their effects on tumor cells [16–18,25]. Fibronectin and a-SMA are commonly used as myofibroblast formation to identify CAFs of ESCC [2,3,21]. Evidence has shown that stromal fibroblasts isolated from cancer tissues such as breast cancer [13] retain the characteristics of CAFs, with high levels of a-SMA expression. As poorly differentiated ESCC has high malignant potential, we isolated and cultured fibroblasts from those special patients, and conformed that the fibroblasts expressed high levels of a-SMA, which is a typical characteristic of CAFs. Moreover, CAFs at low passages cultured *in vitro* retained their original features, which was consistent with the Yu's report [13].

Numerous studies suggested that CAFs could promote tumor invasion and metastasis through EMT [10–14]. Moreover, several reports indicated that radiation to fibroblasts could promote cancer cells invasion and metastasis *in vivo* and *in vitro* [15–20].Our study also showed that irradiated fibroblasts promoted ESCC cells invasion and scattering abilities (a well-characterized behavior activated by HGF/c-Met system [16]), which was consistent with the Patel's report that ionizing radiation enhanced esophageal epithelial cell migration and invasion through a paracrine mechanism involving stromal-derived HGF [20]. And we speculated that X-rays source and γ-ray had some commen effects in process of tumor–stromal interactions to some extent. Furthermore, we found that exposure of ESCC cells to supernatant from irradiated fibroblasts resulted in decreased E-cadherin, increased vimentin *in vitro* and β-catenin was demonstrated to localize to the nucleus in tumor cells with irradiated fibroblasts *in vivo* models. As we known, the most important event of EMT is loss of E-cadherin, which was demonstrated to be a prerequisite for epithelial tumor cell invasion. E-cadherin has dual functions in epithelial cells: as cell–cell adhesion molecule and as negative regulator of the canonical WNT signaling cascade, in particular of its central mediator β-catenin [26]. On the contrary, β-catenin activation increases cell proliferation, directed differentiation and promoted elements of early EMT, including increased *Snail* transcription and reduced E-cadherin expression [27]. Nuclear β-catenin induces a gene expression pattern favoring tumor invasion, and mounting evidence indicates multiple reciprocal interactions of E-cadherin and β-catenin with EMT-inducing transcriptional repressors to stabilize an invasive mesenchymal phenotype of epithelial tumor cells [26]. Recently, Fu L et al. demonstrated that CAFs-secreted Wnt2 could enhance esophageal cancer cell motility and invasiveness by

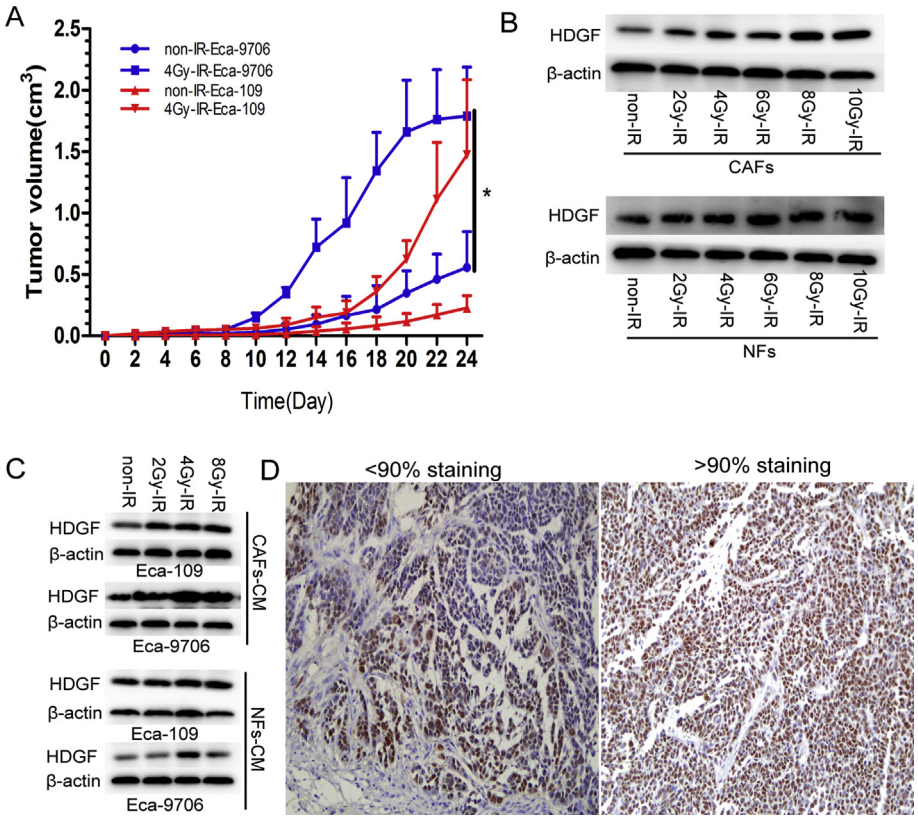

Fig. 4. Irradiated fibroblasts promote HDGF expression of ESCC cells. (A) Tumor growth curves in nude mice after inoculation of ESCC cells with non- or 4Gy-irradiated fibroblasts. Values are the means ± SD (n = 6) of changes in the tumor volumes in each group. *P < 0.05. The expression of HDGF was increased in both irradiated fibroblasts (B) and ESCC cells (C) with them *in vitro* and *in vivo* models (D). Cases with >90% of tumor cells showing nuclear and cytoplasmic positive staining were categorized as level 1 HDGF staining, and the remaining cases as level 0 HDGF staining (Scale bar = 50 μm). Tumor cells showing staining that was equal to or stronger than the staining intensity in endothelial cells were considered positive.

inducing EMT. The expression of β-catenin and E-cadherin was significantly downregulated and β-catenin was translocated into the nucleus [24]. Evidences for EMT were absence of E-cadherin, increased vimentin, β-catenin nuclear relocalization, increased migratory capacity, spindle-shape morphology and loss of polarity and phenotype stable upon removal of inducing stimulus [28]. All the data suggested that irradiated stromal fibroblasts-induced EMT might be the common mechanism underlying the acquisition of metastatic potential in different ESCC subtypes.

HDGF is a new multifunctional protein [29] and involved in numerous biological processes in fibroblasts and cancer cells [5]. HDGF overexpression promoted EMT in cancer progression [30,31] and the expression status of *HDGF* was associated with sensitivity to X-ray irradiation in both cell lines and clinical ESCC [6]. In our study, we first found that X-ray irradiation induced enhanced HDGF expression in fibroblasts and ESCC cells of irradiated group *in vitro* and *in vivo*. HDGF was localized mainly in the nucleus of all the tumor tissues of 24 nude mice. Moreover, the tumor cells adjoining the stromal fibroblasts displayed strong HDGF immunoreactivity, whereas those tumor cells residing in the central part of tumor region expressed a relatively low level of HDGF, which suggested the occurrence of a paracrine effect of fibroblasts on HDGF expression. Besides, ionizing radiation enhances esophageal epithelial cell migration and invasion through a paracrine mechanism involving stromal-derived HGF [20]. HGF contributed to the HDGF-associated aggressive behavior of cancer cells [32]. Our study also showed that irradiated fibroblasts promoted ESCC cells scattering abilities, which was a well-characterized behavior activated by HGF/c-Met system [16]. All the data suggest that radiation to fibroblasts can activate ESCC cells with enhanced HDGF, which was suggested to be a radiosensitive protein of ESCC. In order to make better use of HDGF in clinical, our further studies will investigate the effect of enhanced HDGF on ESCC xenografts during radiotherapy *in vivo*.

In conclusion, our studies showed that irradiated fibroblasts promoted malignant tendency of ESCC, such as invasion, growth and EMT. Meanwhile, it could also enhance HDGF, a radiosensitive protein, expression of ESCC. These may have important clinical implications during radiotherapy of ESCC.

Conflict of interest

None declared.

Acknowledgments

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Transparency document

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References

[1] R. Siegel, J.M. Ma, Z.H. Zou, et al., Cancer statistics, 2014, Ca-Cancer J. Clin. 64 (2014) 9–29.

[2] S.Y. Ha, S.Y. Yeo, Y.H. Xuan, et al., The prognostic significance of cancer-associated fibroblasts in esophageal squamous cell carcinoma, PLoS One 9 (2014) e99955.
[3] Y. Chen, X. Li, H. Yang, et al., Expression of basic fibroblast growth factor, CD31, and alpha-smooth muscle actin and esophageal cancer recurrence after definitive chemoradiation, Tumour Biol. 35 (2014) 7275–7282.
[4] M.H. Barcellos-Hoff, C. Park, E.G. Wright, Radiation and the microenvironment – tumorigenesis and therapy, Nat. Rev. Cancer 5 (2005) 867–875.
[5] C. Bao, J. Wang, W. Ma, et al., HDGF: a novel jack-of-all-trades in cancer, Future Oncol. 10 (2014) 2675–2685.
[6] A. Matsuyama, H. Inoue, K. Shibuta, et al., Hepatoma-derived growth factor is associated with reduced sensitivity to irradiation in esophageal cancer, Cancer Res. 61 (2001) 5714–5717.
[7] S. Yamamoto, Y. Tomita, Y. Hoshida, et al., Expression level of hepatoma-derived growth factor correlates with tumor recurrence of esophageal carcinoma, Ann. Surg. Oncol. 14 (2007) 2141–2149.
[8] P. Cirri, P. Chiarugi, Cancer-associated-fibroblasts and tumour cells: a diabolic liaison driving cancer progression, Cancer Metast. Rev. 31 (2012) 195–208.
[9] J.P. Thiery, H. Acloque, R.Y.J. Huang, et al., Epithelial-mesenchymal transitions in development and disease, Cell 139 (2009) 871–890.
[10] J.P. Coppe, C.K. Patil, F. Rodier, et al., Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor, PLoS Biol. 6 (2008) 2853–2868.
[11] R. Liu, J.Y. Li, K. Xie, et al., FGFR4 promotes stroma-induced epithelial-to-mesenchymal transition in Colorectal cancer, Cancer Res. 73 (2013) 5926–5935.
[12] Y. Luo, L. Lan, Y.G. Jiang, et al., Epithelial-mesenchymal transition and migration of prostate cancer stem cells is driven by cancer-associated fibroblasts in an HIF-1alpha/beta-catenin-dependent pathway, Mol. Cell. 36 (2013) 138–144.
[13] Y. Yu, C.H. Xiao, L.D. Tan, et al., Cancer-associated fibroblasts induce epithelial-mesenchymal transition of breast cancer cells through paracrine TGF-beta signalling, Brit. J. Cancer 110 (2014) 724–732.
[14] B. Zhou, W.L. Chen, Y.Y. Wang, et al., A role for cancer-associated fibroblasts in inducing the epithelial-to-mesenchymal transition in human tongue squamous cell carcinoma, J. Oral Pathol. Med. 43 (2014) 585–592.
[15] M.H. Barcellos-Hoff, S.A. Ravani, Irradiated mammary gland stroma promotes the expression of tumorigenic potential by unirradiated epithelial cells, Cancer Res. 60 (2000) 1254–1260.
[16] K. Ohuchida, K. Mizumoto, M. Murakami, et al., Radiation to stromal fibroblasts increases invasiveness of pancreatic cancer cells through tumor-stromal interactions, Cancer Res. 64 (2004) 3215–3222.
[17] N. Kamochi, M. Nakashima, S. Aoki, et al., Irradiated fibroblast-induced bystander effects on invasive growth of squamous cell carcinoma under cancer-stromal cell interaction, Cancer Sci. 99 (2008) 2417–2427.
[18] K.K.C. Tsai, J. Stuart, Y.Y.E. Chuang, et al., Low-dose radiation-induced senescent stromal fibroblasts render nearby breast Cancer cells radioresistant, Radiat. Res. 172 (2009) 306–313.
[19] C. Chargari, C. Clemenson, I. Martins, et al., Understanding the functions of tumor stroma in resistance to ionizing radiation: emerging targets for pharmacological modulation, Drug Resist. Updat. 16 (2013) 10–21.
[20] Z.S. Patel, K.D. Grugan, A.K. Rustgi, et al., Ionizing radiation enhances esophageal epithelial cell migration and invasion through a paracrine mechanism involving stromal-derived hepatocyte growth factor, Radiat. Res. 177 (2012) 200–208.
[21] C. Zhang, L. Fu, J. Fu, et al., Fibroblast growth factor receptor 2-positive fibroblasts provide a suitable microenvironment for tumor development and progression in esophageal carcinoma, Clin. Cancer Res. 15 (2009) 4017–4027.
[22] X.T. Wang, C.H. Bao, Y.B. Jia, et al., BIIB021, a novel Hsp90 inhibitor, sensitizes esophageal squamous cell carcinoma to radiation, Biochem. Biophys. Res. Commun. 452 (2014) 945–950.
[23] H.C. Chen, Cell-scatter assay, Methods Mol. Biol. 294 (2005) 69–77.
[24] L. Fu, C.Y. Zhang, L.Y. Zhang, et al., Wnt2 secreted by tumour fibroblasts promotes tumour progression in oesophageal cancer by activation of the Wnt/beta-catenin signalling pathway, Gut 60 (2011) 1635–1643.
[25] N. Erez, M. Truitt, P. Olson, et al., Cancer-Associated fibroblasts are activated in incipient neoplasia to orchestrate tumor-promoting inflammation in an NF-kappaB-dependent manner, Cancer Cell. 17 (2010) 135–147.
[26] O. Schmalhofer, S. Brabletz, T. Brabletz, E-cadherin, beta-catenin, and ZEB1 in malignant progression of cancer, Cancer Metastasis Rev. 28 (2009) 151–166.
[27] A. Giangreco, L. Lu, C. Vickers, et al., Beta-Catenin determines upper airway progenitor cell fate and preinvasive squamous lung cancer progression by modulating epithelial-mesenchymal transition, J. Pathol. 226 (2012) 575–587.
[28] M. Zeisberg, E.G. Neilson, Biomarkers for epithelial-mesenchymal transitions, J. Clin. Invest. 119 (2009) 1429–1437.
[29] J. Zhao, H. Yu, L. Lin, et al., Interactome study suggests multiple cellular functions of hepatoma-derived growth factor (HDGF), J. Proteomics 75 (2011) 588–602.
[30] S.C. Chen, M.L. Kung, T.H. Hu, et al., Hepatoma-derived growth factor regulates breast cancer cell invasion by modulating epithelial–mesenchymal transition, J. Pathol. 228 (2012) 158–169.
[31] H.E. Tsai, G.S. Liu, M.L. Kung, et al., Downregulation of hepatoma-derived growth factor contributes to retarded lung metastasis via inhibition of epithelial-mesenchymal transition by systemic POMC gene delivery in Melanoma, Mol. Cancer Ther. 12 (2013) 1016–1025.
[32] A. Zhang, W. Long, Z. Guo, et al., Downregulation of hepatoma-derived growth factor suppresses the malignant phenotype of U87 human glioma cells, Oncol. Rep. 28 (2012) 62–68.