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ORIGINAL ARTICLE

Hepatocellular carcinoma cells cause different responses in expressions of cancer-promoting genes in different cancer-associated fibroblasts

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Abstract Cancer-associated fibroblast (CAF) is one of the most crucial components of the tumor microenvironment to promote the invasiveness of cancer cells. The interactions between cancer cells and CAFs are bidirectional. Our recent study showed that up-regulations of chemokine (C-C motif) ligand 2 (*CCL2*), chemokine (C-C motif) ligand 26 (*CCL26*), interleukin 6 (*IL6*), and lysyl oxidase-like 2 (*LOXL2*) genes in cancer cells were parts of the common effects of CAFs on hepatocellular carcinoma (HCC) cells to promote proliferation, migration and invasion of cancer cells. However, the subject of how HCC cells to influence the gene expressions of CAFs still needs to be clarified. The purpose of this study was to investigate this issue. Two human HCC (HCC24/KMUH, HCC38/KMUH) and two human CAF cell lines (F26/KMUH, F28/KMUH) were studied. Influence of HCC38/KMUH cancer cells on differential expressions of genes in F28/KMUH CAFs was detected by microarray to select target genes for further analysis. Both HCC cell lines increased proliferation (all $p < 0.005$) and migration (all $p < 0.0001$) of two CAF cell lines. HCC24/KMUH cancer cells had stronger ability to promote migration of F26/KMUH CAFs than HCC38/KMUH cancer cells did ($p < 0.0001$). Eleven up-regulated cancer-promoting genes, including apelin (APLN), *CCL2*, *CCL26*, fibroblast growth factor 1 (FGF1), fibroblast growth factor 2 (FGF2), *IL6*, mucin 1 (MUC1), *LOXL2*, platelet-derived growth factor alpha polypeptide (PDGFA), phosphoglycerate kinase 1 (PGK1), and vascular endothelial growth factor A (VEGFA) detected by microarray showed good correlation with results of quantitative reverse transcriptase-polymerase chain

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reaction study. Among these genes, HCC24/KMUH cancer cells had same tendency of effects on differential expressions of genes in F28/KMUH CAFs as HCC38/KMUH cancer cells did. However, the responses of F26/KMUH CAFs to different HCC cell lines were variable. Only PGK1 gene was consistently up-regulated and PDGFA gene was consistently down-regulated caused by both HCC cell lines in F26/KMUH CAFs. Besides *PGK1* gene, HCC38/KMUH cancer cells only up-regulated *APLN*, *LOXL2*, and *VEGFA* genes and HCC24/KMUH cancer cells only up-regulated *FGF2* gene in F26/KMUH CAFs. In conclusion, HCC cells can promote proliferation and migration of CAFs. However, the impact of HCC cells on differential expressions of cancer-promoting genes in CAFs is influenced by the characteristics of CAFs. This implies that blocking single or several particular cancer-promoting genes in CAFs is unable to become a common stratagem for the treatment of HCC.

Introduction

Invasion and metastasis of malignancy are determined by the characteristics of cancer cells and the interactions between the cancer cells and the tumor microenvironment. Cancer-associated fibroblast (CAF) is one of the most crucial components of the tumor microenvironment to promote the growth and invasion of cancer cells [1—5]. The interactions between cancer cells and CAFs are bidirectional and are initiated from secretion of soluble factors from cancer cells to enhance the ability of CAFs to secrete a variety of tumor-promoting factors [3]. Hepatocellular carcinoma (HCC) is the most common primary liver cancer and ranks globally as the third to fourth leading cause of cancer-related death [6,7]. Understanding the HCC—CAF interactions may help us to target the tumor microenvironment and thus to improve the prognosis of HCC [4]. Our recent study showed that up-regulations of chemokine (*C-C motif*) ligand 2 (*CCL2*), C-C motif ligand 26 (*CCL26*), interleukin 6 (*IL6*), and lysyl oxidase-like 2 (*LOXL2*) genes in cancer cells were parts of the common effects of CAFs on HCC cells to promote proliferation, migration, and invasion of cancer cells [8]. Although lysophosphatidic acid secreted from HCC cells had been demonstrated to accelerate HCC progression by recruiting peritumoral tissue fibroblasts and promoting their transdifferentiation into CAFs [5], the subject of how HCC cells to influence the gene expressions of CAFs still need to be clarified. Moreover, the characteristics of CAFs have significant individual/intrinsic differences [9]. This suggests that the responses of different CAFs to different HCC cells stimulations may be variable. The purpose of this study was to investigate whether different human CAF cell lines had different responses to different human HCC cell lines. All gene names are according to the official symbols from the HUGO Gene Nomenclature Committee.

Methods

Cell lines

Two CAF cell lines (F26/KMUH, F28/KMUH) newly established from patients with HCC in our institution and two human HCC cell lines (HCC24/KMUH, HCC38/KMUH) used in our previous studies [10—12] were investigated. CAF cell lines were verified by positive stain for fibroblast activation protein (ENZO Life Sciences International, Inc., Butler Pike, Plymouth Meeting, PA, USA), α-smooth muscle actin (Sigma-Aldrich, St. Louis, MO, USA) and chemokine (C-X-C motif) ligand 12 (CXCL12; R & D Systems, Inc., Minneapolis, MN, USA). Both CAF cell lines were also verified to have capacities to penetrate the Matrigel. All procedures to establish these cell lines were approved by the Institutional Review Board of our hospital and patients were given informed consent. All cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. The serum-containing culture medium consisted of 10% fetal bovine serum, 90% DME/HIGH glucose, supplemented with 20 mM L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin (HyClone, Logan, UT, USA).

Influence of HCC cells on proliferation of CAFs

CAFs from each cell line were seeded in three 24-well cell culture insert companion plates (18 wells/plate, 1×10^5 cells/well). Cancer cells from each cell line were seeded in 36 culture inserts (2×10^5 cells/insert) with transparent polyethylene terephthalate membrane (pore size: 0.4 μm, BD Falcon, Cell Culture Inserts, BD Biosciences, Mississauga, Ontario, Canada) using two new 24-well companion plates. Both CAFs and cancer cells were incubated with serum-containing medium for 24 hours, then all medium was replaced with serum-free medium and the inserts were transferred to CAFs containing wells. The cells were incubated for another 48 hours. Then the inserts were removed and CAFs in each well were analysis. The premixed WST-1 cell proliferation reagent (Clontech Laboratories, Inc., A Takara Bio Company, Mountain View, CA, USA) was applied. The experimental procedures were carried out following the manufacturer's protocols. The cells were incubated with reagent for 4 hours at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. After that, 0.1 mL suspension in each well was transferred to 96-well plate for automated microplate reader (MRX, Dynex Technologies, Inc., Chantilly, VA, USA) analysis. The absorbance was measured at 450 nm wavelength (reference wavelength, 630 nm).