BioMed Research Journal
BMRJ, 6(1): 485-490
www.scitcentral.com

ISSN: 2578-8892

Original Research Article: Open Access

Phenotypic Changes and Cell Migration of Bile Duct Cancer Cells Induced by Mesenchymal Cells

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Received September 08, 2021; Revised September 11, 2021; Accepted September 14, 2021

ABSTRACT

Bile duct cancer (biliary tract cancer) is one of the most high-grade malignancies because of its invasiveness and metastasis. Local aggressive invasiveness of the cancer is thought to depend on microenvironments with cancer cells and stromal cells. We analyzed phenotypic changes and cell migration of the TFK-1 human extra hepatic bile duct cancer cells, co-cultured with MSC-43 human mesenchymal stem cells. When co-cultured with MSC-43 cells, the TFK-1 cells morphologically showed epithelial mesenchymal transition (EMT), and immunohistochemically decreased E-cadherin expression. Migration assay demonstrated that the TFK-1 cells co-cultured with MSC-43 cells showed significantly higher migration rate than the TFK-1 cells only (2.48 vs 1.82, $p<0.001$). Transwell migration assay revealed that the TFK-1 cells indirectly co-cultured with MSC-43 cells exhibited higher migration rate than the TFK-1 cells only (3.27 vs 1.67, $p<0.005$). In conclusion, the stromal mesenchymal cells are thought to play an important role of phenotypic changes and cell migration of human bile duct cancer.

Keywords: Bile Duct Cancer, Stromal Mesenchymal Cells, Cell Migration, Morphology

INTRODUCTION

Extra hepatic bile duct cancer (biliary tract cancer) is recognized as one of the most aggressive malignancies because of its frequent metastasis and recurrence [1-3]. Most of the patients are usually treated at an advanced stage, and the prognosis remains poor despite the development of recent diagnosis and treatment. Therefore, the bile duct cancer is the second most lethal malignancy, following the pancreatic cancer. Local invasiveness of the extra hepatic bile duct cancer has not yet been analyzed morphologically, while status of the histopathology of lymph node metastasis has been reported previously [4].

Here, we focus on the co-culture of human bile duct cancer cells with human mesenchymal stem cells, and describe phenotypic changes and cell migration of the bile duct cancer.

MATERIALS AND METHODS

Cells

TFK-1 is a human cell line derived from extrahepatic bile duct carcinoma, obtained from the RIKEN BRC through the National Bio-Resource Project of MEXT (Ministry for Education, Culture Sports, Science and Technology), Japan [5]. The TFK-1 cells cultured in RPMI 1640 medium with 10% FBS and 1% penicillin-streptomycin. MSC-43 is derived from human mesenchymal stem cells, obtained from the RIKEN BRC through the National Bio-Resource Project of MEXT, Japan. The MSC-43 cells cultured in DMEM with 10% FBS and 3ng/mL basic fibroblast growth factor (bFGF).

Direct co-culture of TFK-1 cells and MSC-43 cells

When co-cultured with MSC-43 cells, the TFK-1 cells were cultured in DMEM, and grew up well on the monolayer plate **(Figures 1A and 1B)**. Immunostainings of E-cadherin (epithelial marker) and vimentin (mesenchymal marker) were performed using Leica BOND-MAX (Leica Biosystems, Nußloch, Germany).

Indirect co-culture of TFK-1 cells and MSC-43 cells

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Citation: Ogasawara H, Yoshizawa T, Kubota S, Goto S, Morohashi S, et al. (2022) Phenotypic Changes and Cell Migration of Bile Duct Cancer Cells Induced by Mesenchymal Cells. BioMed Res J, 6(1): 485-490.

TFK-1 cells were cultured on the monolayer plate, while MSC-43 cells were cultured on the upper transwell insert **(Figure 1C)**.

Migration assay

The cultured cells on the monolayer plate were scratched with a cell scraper, and no cellular area on the plate was made **(Figure 1D)**. Co-culture was maintained for 6 days. The experiments were performed at least triplicated. Migration rate [μm/h] is calculated by the formula: Width (Day 0) - Width (Day N) x 0.5 / 24 x N.

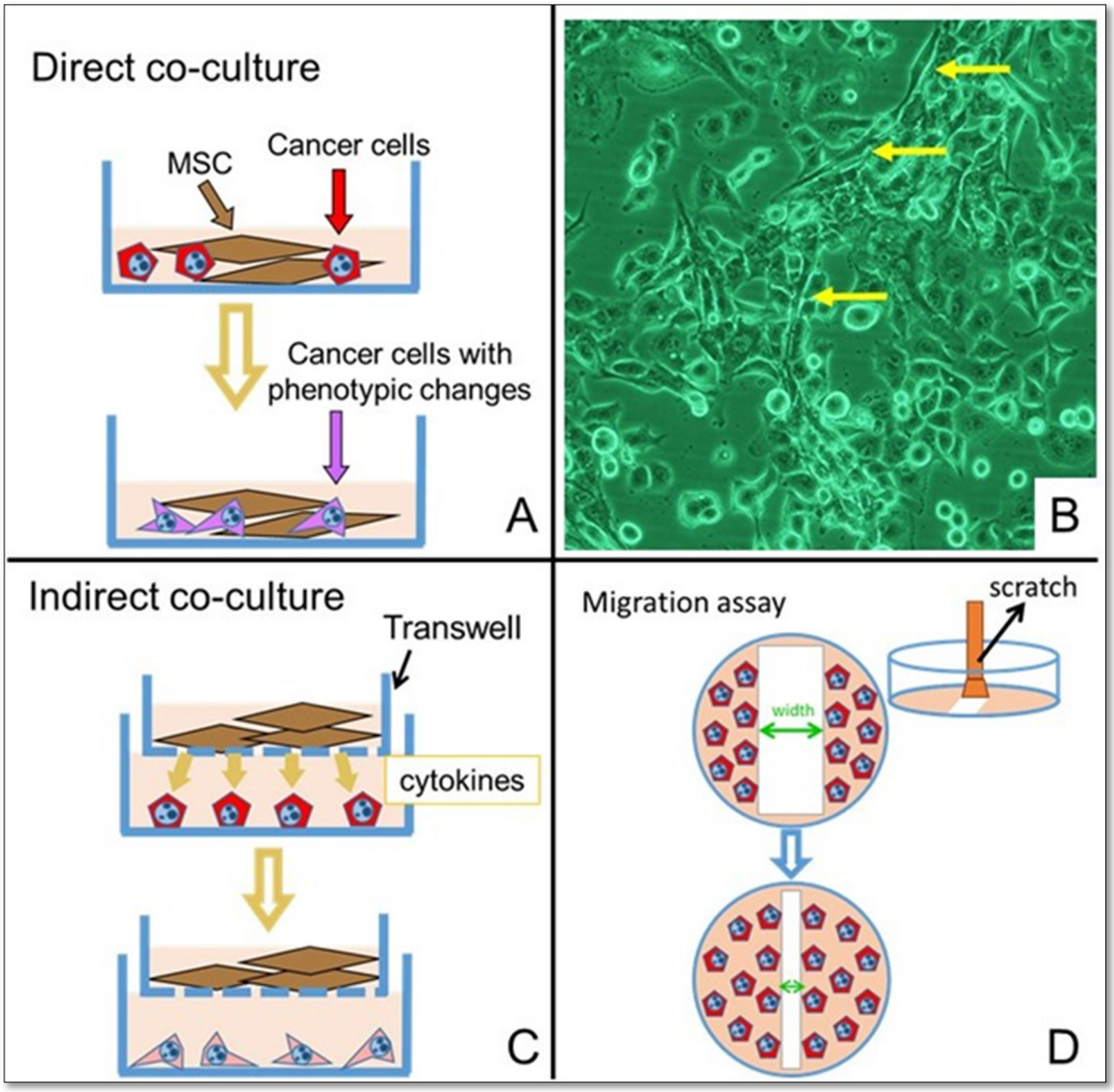

Figure 1. TFK-1 human extrahepatic bile duct cancer cells. Both TFK-1 cells and MSC-43 cells are cultured on the monolayer culture plate (A: top left, direct co-culture). Polygonal TFK-1 cells are co-cultured with MSC-43 human mesenchymal stem cells (arrows, spindle cells) (B: top right, monolayer culture). TFK-1 cells are cultured on the monolayer plate, while MSC-43 cells are cultured on the upper transwell insert (C: bottom left, indirect co-culture). Migration assay is shown schematically (B: bottom right).

RESULTS

Direct co-culture of TFK-1 cells and MSC-43 cells

When co-cultured with MSC-43 cells, the TFK-1 cells grew up well on the monolayer plate **(Figure 2)**. Morphologically, the polygonal TFK-1 cells formed monolayer nests, while

some of the cells become spindle in shape. Immunohistochemically, the cell membrane of polygonal TFK-1 cells expressed E-cadherin, an epithelial marker, while the cytoplasm of MSC-43 cells showed vimentin, a mesenchymal marker. The spindle TFK-1 cells decreased E-cadherin expression, and exhibited weak vimentin expression **(Figure 2D)**, indicating epithelial mesenchymal transition (EMT).

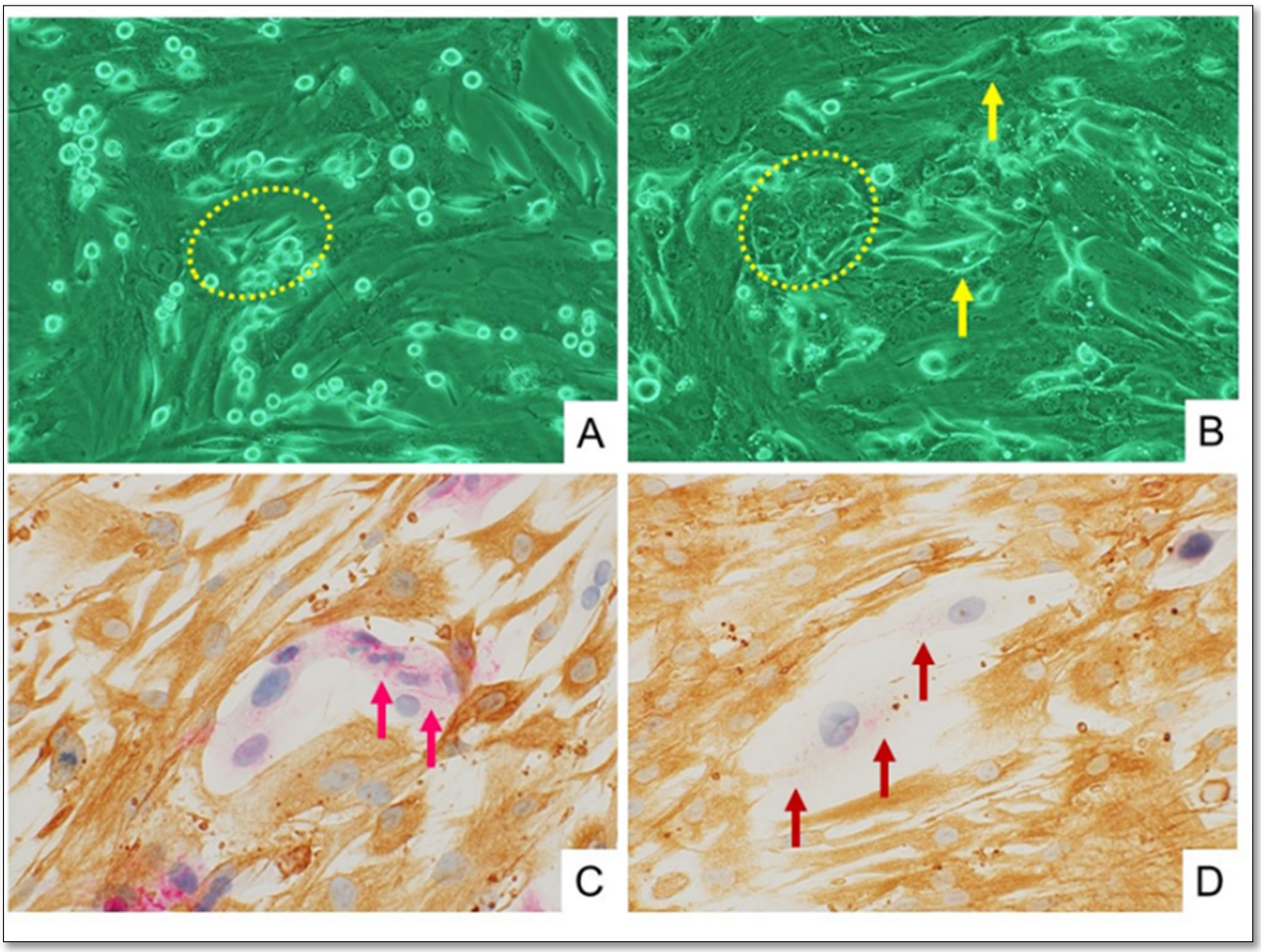

Figure 2. Direct co-culture of TFK-1 cells and MSC-43 cells. Polygonal TFK-1 cells are seen (dotted circle), surrounded by spindle MSC-43 cells (A: top left, Day 1). TFK-1 cells form monolayer nests (dotted circle), while some of the cells become spindle in shape (arrows) (B: top right, Day 2). TFK-1 cells express E-cadherin (arrows, red at cell membrane), while MSC-43 cells express vimentin (brown in cytoplasm) (C: bottom left, Day 2, immunohistochemical staining). TFK-1 cells weakly express vimentin (arrows, red at cell membrane) (D: bottom right, Day 4, immunohistochemical staining).

Cell migration of TFK-1 cells directly co-cultured with MSC-43 cells

The TFK-1 cells were co-cultured with MSC-43 cells on the monolayer plate. Migration assay demonstrated cell migration of both TFK-1 cells and MSC-43 cells on the monolayer plate **(Figures 3A, 3B, and 4A)**. TFK-1 cells co-cultured with MSC-43 cells showed significantly higher migration rate than the TFK-1 cells only (2.48 ± 0.32 vs 1.82 ± 0.32, $p<0.001$).

Cell migration of TFK-1 cells indirectly co-cultured with MSC-43 cells

TFK-1 cells were cultured on the monolayer plate, while MSC-43 cells were cultured on the upper transwell insert. Migration assay demonstrated that TFK-1 cells indirectly co-cultured with MSC-43 cells showed significantly higher migration rate than the TFK-1 cells only (3.27 ± 0.47 vs 1.67 ± 0.68, $p<0.05$) **(Figures 3C, 3D, and 4B)**.

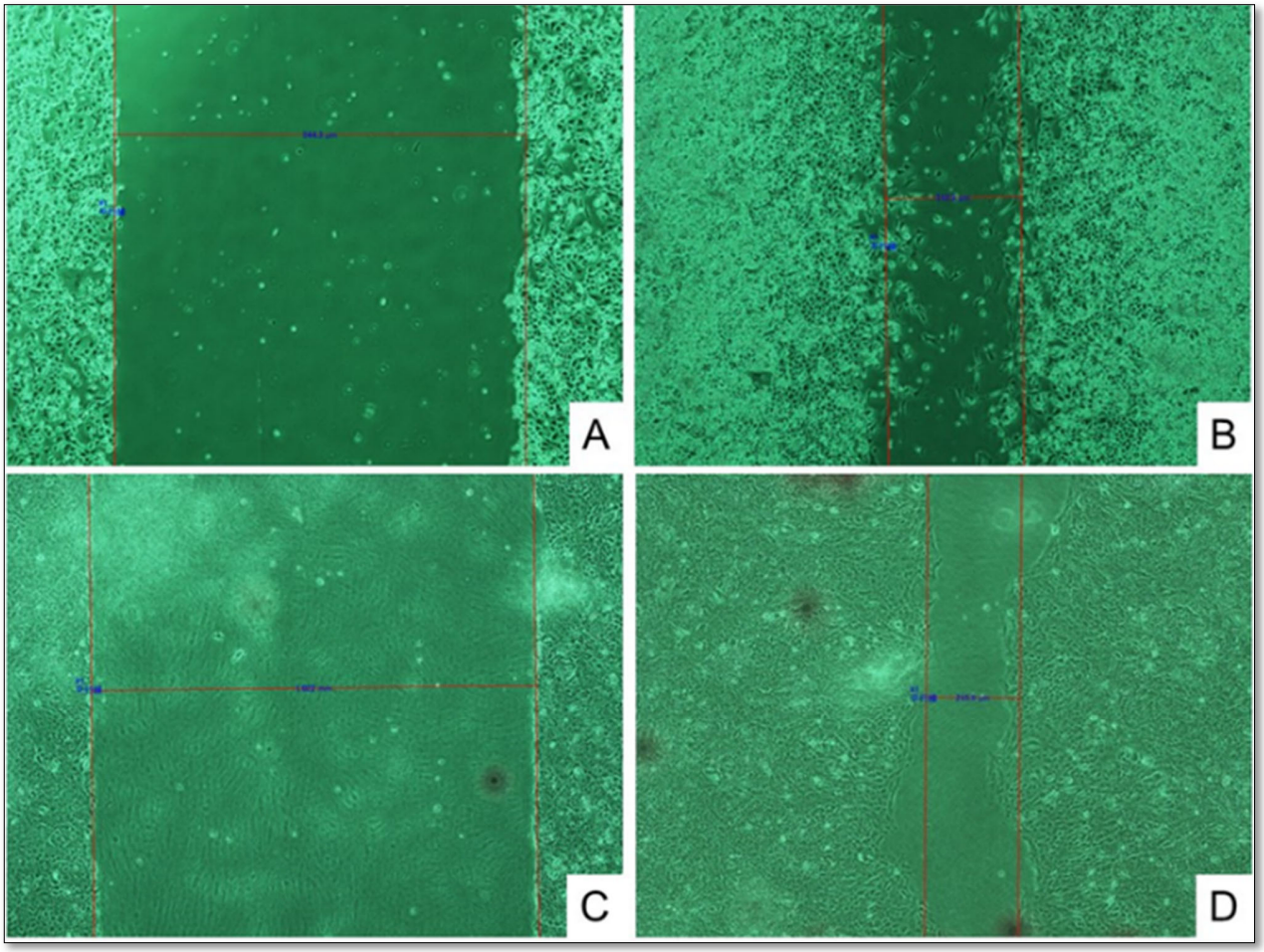

Figure 3. Migration assay of TFK1 cells. Direct co-culture of TFK-1 cells and MSC-43 cells show no cell area on Day 0 (A: top left), and narrowed width on Day 5 (B, top right). Indirect co-culture of TFK1 cells with MSC-43 cells show no cell area on Day 0 (D: bottom left), and narrowed width on Day 5 (D, bottom right).

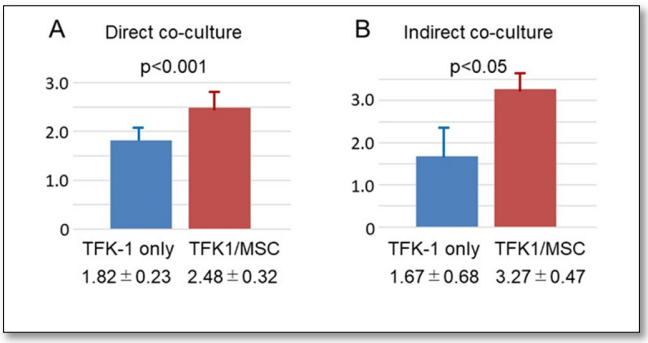

Figure 4. Results of migration assay of TFK-1 cells. Migration rate [μm/h] is calculated by the formula: Width (Day 0) - Width (Day N) x 0.5 / 24 x N. Direct co-culture of TFK-1 cells and MSC-43 cells is higher than that of TFK-1 cells only (2.48 vs 1.82, $p<0.001$) (A: left). Indirect co-culture of TFK-1 cells with MSC-43 CELLS is higher than that of TFK-1 cells only (3.27 vs 1.67, $p<0.05$) (B: right).

DISCUSSION

We describe morphological and cell biological characteristics of biliary cancer. When co-cultured with MSC-43 cells, the TFK-1 human bile duct cancer cells showed morphological changes indicating EMT, and high cell migration rate.

The most extra hepatic bile duct cancers are malignancies arising from the bile duct epithelium, and exhibit one of the highly aggressive malignancies [6-8]. We demonstrated that invasive growth with high-grade tumor budding was thought to be associated with aggressiveness of the pancreato-biliary cancer [9]. However, histopathological mechanisms of local invasiveness in the cancer microenvironment have not yet clarified, while the molecular/genetic profiles of cancer cells have been shown recently.

Microenvironment is important when the cancer cells grow up in their native tissues/organs. Malignancy in cancers is characterized by invasion and metastasis that are closely

associated with interaction between cancer cells and non-cancerous stroma [10,11]. The cancer cells interact with adjacent epithelial cells, tumor-associated matrix constituents, and stromal/inflammatory cells [12,13]. However, mechanisms of cancer cell migration/invasion have not well understood. Our study is the first to demonstrate histopathological findings of cancer cell migration using direct/indirect co-culture with stromal mesenchymal cells.

EMT is one of important steps in cancer invasion and metastasis, and involve related behavioral patterns that lead cells to remove intercellular bonds digest basement membranes, rearrange extracellular matrix, and navigate through that matrix [14]. The cancer cells in EMT processes are characterized by the loss of epithelial factors, including cytokeratin and E-cadherin, and the up regulated expression of mesenchymal factors, including vimentin and N-cadherin [15-18]. Our morphological/immune histochemical study revealed that the TFK-1 human bile duct cancer cells showed EMT when co-cultured with MSC-43 cells. Moreover, TFK-1 cells co-cultured with MSC-43 cells directly/indirectly showed the significantly higher cell migration. The results indicated that indirect interactions such as cytokines were thought to associate with the migration and local invasion of the cancer cells.

In conclusion, we demonstrate histopathological characteristics of the extra hepatic bile duct cancer cells co-cultured with mesenchymal cells. The stromal mesenchymal cells are thought to play an important role of phenotypic changes and cell migration of human bile duct cancer.

ACKNOWLEDGEMENTS

This study was supported by JSPS KAKENHI, Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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