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New Therapeutic Strategy for Intractable Pancreatic Cancer and Its Fundamental Research

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is now the fifth leading cause of cancer in Japan, the United States, and Europe, with an overall 5-year survival rate of less than 5%. One reason for this poor prognosis is the tendency of pancreatic cancer cells to invade adjacent tissues and to metastasize even at early stage.

A high percentage of PDACs also overexpress several growth factors and their receptors, including the epidermal growth factor receptor, epidermal growth factor, human epidermal growth factor receptor 2, vascular endothelial growth factor (VEGF) receptor, insulin-like growth factor receptor, and fibroblast growth factor (FGF) receptor (FGFR).

The multiple alterations in oncogenes and tumor suppressor genes in conjunction with the overexpression of mitogenic growth factors and their receptors may contribute to the biological aggressiveness of pancreatic cancers and to the formation of the abundant stroma that characterize them.

Keratinocyte growth factor (KGF) is a member of the FGF group of heparin-binding polypeptides which was originally isolated from human embryonic lung fibroblasts. The actions of KGF are dependent on its binding to a specific cell-surface KGF receptor (KGFR), also known as FGFR type II (FGFR2 IIIb).

KGF is expressed in a variety of tissues, including the lung, prostate, mammary gland, digestive tract, bladder, and skin, and is implicated in organ development and homeostasis. Moreover, KGF-expressing transgenes exhibit pancreatic ductal hyperplasia, and KGF mRNA levels are elevated in PDAC. Although we have previously reported that KGF and KGFR are overexpressed in both pancreatic cancer cells and the adjacent pancreatic parenchyma[1], the potential roles of KGF and KGFR in PDAC are still poorly understood. We have previously reported that co-expression of KGF and KGFR in PDAC is associated with venous invasion, enhanced VEGF-A expression, and poor prognosis[2]. Matrix metalloproteinase (MMP)-9 participates in the degradation of type IV collagen; therefore, in the present study, we investigated the expression and correlation of KGF, KGFR, and MMP-9 in human pancreatic cancer cell lines and tissues.

Methods

Expression of KGF, KGFR, and MMP-9 were examined in 8 human pancreatic cancer cell lines. Immunohistochemical analyses were performed with antibodies against KGF, KGFR, VEGF-A, or MMP-9 in 53 cases of pancreatic cancer. Moreover, the correlation of clinicopathological factors with KGF, KGFR, VEGF-A, and MMP-9 in pancreatic cancer cases was evaluated.

We also examined whether exogenous or endogenous KGF up-regulates VEGF-A or MMP-9 expression in pancreatic cancer cell lines. Moreover, to establish a better link between the KGF/KGFR pathway and VEGF-A or MMP-9 expression, we performed experiments using short hairpin (sh)-KGF and sh-KGFR. The effects of KGF on the migration and invasiveness in human pancreatic cancer cells were studied in cell migration and invasion assays.

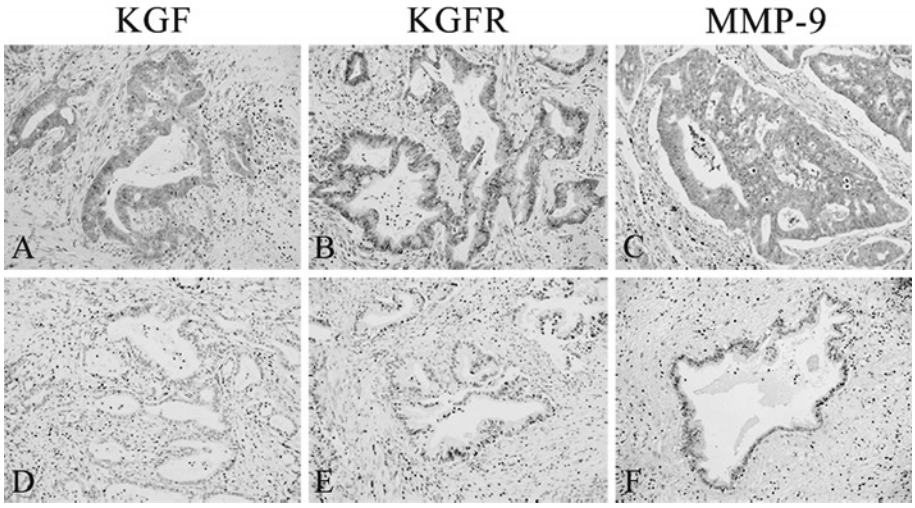

Fig. 1 Immunohistochemical analyses of KGF, KGFR, and MMP-9 in human pancreatic cancer tissues

A–C: Characteristic staining patterns of KGF, KGFR, and MMP-9 in cases of human pancreatic cancer. **A**: KGF immunoreactivity was detected in the cytoplasm of cancer cells and stromal fibroblasts. **B**: KGFR immunoreactivity was detected in the cytoplasm and cell membrane of cancer cells. **C**: MMP-9 immunoreactivity was detected in the cytoplasm of cancer cells and stromal fibroblasts. **D–F**: Cases negative for KGF, KGFR and MMP-9. Immunohistochemistry, KGF (A and D), KGFR (B and E), and MMP-9 (C and F); original magnification, ×200.

Results and Discussion

KGFR and MMP-9 mRNA were detected in all pancreatic cancer cell lines. In contrast, KGF mRNA was detected in 7 of 8 pancreatic cancer cell lines. KGF, KGFR, and MMP-9 were localized in pancreatic cancer cells in 18 (34.0%), 22 (41.5%), and 22 of the 53 cases (41.5%), respectively **(Fig. 1)**. Venous invasion was significantly correlated with the expression of KGF, KGFR, and MMP-9. Furthermore, the co-expression of KGF and KGFR was significantly correlated with venous invasion, MMP-9 expression, and poor prognosis. In addition, the expression KGF and MMP-9 were significantly related to liver metastasis.

Exogenous KGF increased VEGF-A and MMP-9 expression in MIA PaCa-2 cells, and PANC-1 cells stably transfected to overexpress KGF exhibited increased VEGF-A expression.

Moreover, transient transfection of sh-KGFR in MIA PaCa-2 cells (KGF-negative, KGFR-positive) reduced the stimulatory effect of exogenous KGF on VEGF-A expression, and sh-KGF transfection in KLM-1 cells (KGF-positive, KGFR-positive) reduced VEGF-A and MMP-9 expression in the cells. Exogenous KGF significantly enhanced the migration and invasion of MIA PaCa-2 cells.

Taken together, these findings suggest that KGF synthesized by pancreatic cancer plays an important role in enhancing venous invasion in pancreatic cancer, in part, by up-regulating the expression of VEGF-A or MMP-9 or both. Because venous invasion indicates a poor prognosis, our findings also suggest that KGF and KGFR are important targets that could lead to novel therapeutic strategies for prolonging the survival of patients with pancreatic cancer.

References

1. Ishiwata T, Friess H, Buchler MW, Lopez ME, Korc M: Characterization of keratinocyte growth factor and receptor expression in human pancreatic cancer. Am J Pathol 1998; 153: 213–222.
2. Cho K, Ishiwata T, Uchida E, et al.: Enhanced expression of keratinocyte growth factor and its receptor correlates with venous invasion in pancreatic cancer. Am J Pathol 2007; 170: 1964–1974.