

CLINICAL STUDY

Increase in the circulating level of hepatocyte growth factor in pancreatic cancer patients

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Abstract: *Objective:* Hepatocyte growth factor (HGF) has been reported the cause of many biological events, including cell proliferation, invasiveness, morphogenesis, and angiogenesis. Elevated HGF content in tumor tissue was reported to predict a more aggressive biology in breast and gastric cancer patients.

Materials and methods: Eighty patients with invasive pancreatic cancer investigated. Venous blood samples were collected before the surgery. Sera were obtained by centrifugation and stored at -70 °C until assayed. The control group created from healthy individuals. Serum concentrations of soluble HGF were measured by the quantitative sandwich enzyme immunoassay technique.

Results: The mean value of serum soluble HGF in patients with invasive pancreatic cancer was 497.2±53.8 pg/ml and that of control group was 53.6±7.5 pg/ml and the difference was significant ($p < 0.001$).

Conclusion: The serum levels of soluble HGF might reflect the severity of invasive pancreatic cancer and deserve further evaluation (Tab. 2, Ref. 19). Full Text (Free, PDF) www.bmj.sk.

Key words: hepatocyte growth factor, invasive, pancreatic cancer.

Pancreatic ductal carcinoma is one of the most fatal cancers showing a high potential for invasive activity and early recurrence with high recurrence rates (1–3). Even with the introduction of recent advances in imaging diagnostics such as computed tomographic scan and ultrasonography, most patients with pancreatic ductal carcinoma are diagnosed at an advanced stage, such as stage III or IV, and the tumors are usually unresectable (2). Even in respectable cases, the prognosis of patients with pancreatic ductal carcinoma is very poor (3, 4). We are thus trying to identify useful prognostic factors other than the disease stage to predict the risk of disease recurrence more accurately.

Hepatocyte growth factor, which is known to be identical to scatter factor, has been reported the cause of many biological events, including cell proliferation (5), movement (6), invasiveness (7), morphogenesis (8) and angiogenesis (9). HGF is found in many organs, including the mammary gland, lung, kidney, and liver (10, 11). Elevated hepatocyte growth factor content in tumor tissue was reported to predict a more aggressive biology in non-small cell lung cancer patients (12). However, there is still limited knowledge about the role of hepatocyte growth factor in pancreatic cancer. The evaluation of the possible outcome of the patient with pancreatic cancer is important for planning optional treatment. Because no prognostic factor can determine the whole

status of a patient with pancreatic cancer, the physician must consider all available prognostic data.

This study was designed with the aim to investigate any correlation between the circulating hepatocyte growth factor and the clinicopathologic variables evaluate the possible prognostic significance of the circulating hepatocyte growth factor in pancreatic cancer.

Materials and methods

90 patients who underwent resections for primary ductal carcinoma of the pancreas at the II. General Surgery Division, Haseki Training Hospital from October 2007 to December 2008 were analyzed for this study. There were 54 males and 36 females of ages ranging from 46 to 78 years (median, 59.5 years).

Preoperative diagnostic imaging examinations, ultrasonography, computed tomographic scan, magnetic resonance imaging study, and angiography were done for all patients. 83 patients initially received curative resection of the tumor whereas 7 patients received noncurative surgery.

Tumor stages were defined based on the pathologic tumor-node-metastasis (pTNM) classification (13).

Venous blood samples were collected before the surgery. Sera were obtained by centrifugation and stored at -70 °C until assayed.

Measurement of Hepatocyte Growth Factor (Principle of the Assay)

This assay (R&D Systems) employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody

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Tab. 1. Serum concentrations of HGF in control group.

	n	mean value of HGF (pg/ml)
Control group	80	53.6±7.5

specific for hepatocyte growth factor has been precoated onto a microplate.

Standards and samples are pipetted into the wells and any hepatocyte growth factor present is bound by the immobilized antibody. After washing away any bound substances, an enzyme-linked polyclonal antibody specific for HGF is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of hepatocyte growth factor bound in the initial step. The color development is stopped and the intensity of the color is measured. The minimum detectable dose of hepatocyte growth factor is typically <40 pg/ml (14).

Statistical analysis

The Student's *t* test was used to assess the significance of difference in the levels of hepatocyte growth factor between the patient group and control group. The following clinicopathologic variables were first entered into the univariate analysis by Student's *t* test or ANOVA test: age, location of tumor, pTNM staging, lymph node status, distant metastases status, histologic differentiation, lymphatic invasion, venous invasion. These clinicopathologic variables were then assessed by the multiple linear regression method. $p < 0.05$ was accepted as significant. Results in pg/ml were expressed as the mean \pm SD.

Results

The mean value of serum soluble hepatocyte growth factor in patients with pancreatic cancer was 497.2 ± 53.8 pg/ml and that of control group was 53.6 ± 7.5 pg/ml and the difference was significant ($p < 0.001$). Furthermore (Tab. 1), there were significantly higher serum levels of soluble hepatocyte growth factor in patients with advanced tumor staging ($p < 0.001$), in patients with more advanced lymph node status ($p < 0.001$), in patients with distant metastases ($p < 0.001$). In multiple linear regression method, pTNM staging ($p < 0.001$) seemed an independent factor regarding the significant higher serum levels of soluble hepatocyte growth factor.

Discussion

To establish effective therapeutic modalities for pancreatic ductal carcinoma, precise assessment of factors affecting tumor progression and patient prognosis is crucial. Although a conventional TNM staging system, tumor progression, lymph node involvement, and distant metastasis, is useful for the stratification of patients with pancreatic ductal carcinoma, poor outcome of patients has been reported even the low-stage (I and II) group (1, 2). Therefore, prognosticators and the accumulation of statis-

Tab. 2. Serum concentrations of HGF in relation to clinicopathologic variables.

Factors	n	mean value of HGF (pg/ml)	p
Age (y)			
≤60	26	423.6±78.5	0.435
>60	54	409.1±54.9	
Gender			
Male	54	436.7±27.4	0.581
Female	36	441.8±31.8	
Location of tumor			
Head	58	485.2±51.5	0.512
Body/tail	22	475.9±49.2	
T (pTNM)			
pT1	0		<0.001
pT2	19	456.2±34.7	
pT3	33	489.1±45.3	
pT4	28	502.9±26.7	
Lymph node metastasis			
Present	69	513.7±19.2	<0.001
Absent	11	476.5±31.7	
Stage (pTNM)			
I	0		<0.001
II	9	423.6±16.8	
III	25	458.1±23.9	
IV	46	501.6±20.4	
Histologic differentiation			
Well differentiation	29	429.8±30.5	<0.001
Moderately differentiation	46	467.1±25.7	
Poorly differentiation	5	495.2±16.7	
Lymphatic invasion			
Present	73	499.7±18.3	<0.001
Absent	7	421.4±43.1	
Venous invasion			
Present	28	487.4±34.8	<0.001
Absent	52	443.7±29.1	

tical analyses are needed to develop an effective stratification system of pancreatic ductal carcinoma.

This study was conducted to evaluate the correlation of circulating soluble hepatocyte growth factor with clinicopathologic variables and its possible prognostic value with the hope to find additional meaningful information for making a treatment decision. In this study, serum was chosen for measurement of hepatocyte growth factor because serum is easily available and sufficient for an objective analysis. The data could be achieved preoperatively and could be useful to an optimal preoperative planning. This method for measurement of hepatocyte growth factor is feasible, reproducible, inexpensive, and widely available; the serum levels of hepatocyte growth factor have already been shown to be of prognostic value in other malignancy (12).

Hepatocyte growth factor is found to be involved in carcinogenesis. Jeffers et al (15) reported that cotransfection of hepatocyte growth factor and c-met was able to induce morphologic transformation in vitro and tumorigenicity in vivo in a nontumorigenic mouse cell line C127. In the bladder cell line NBT-II, trans-

fection of hepatocyte growth factor upgraded the invasive phenotype and growth rate of these cells (16).

One study was reported that hepatocyte growth factor expression may exist in epithelial cells and mesenchymal compartments (17). Other study was reported that Hepatocyte growth factor receptor is widely distributed in various epithelial cells including tumor cells but obviously not in mesenchymal cell (18). On the other hand, hepatocyte growth factor production was found in the stromal component (17). Because it has been reported that hepatocyte growth factor is a modulator of epithelial cell proliferation and motility for a broad spectrum of cell types (19), it is tempting to speculate that hepatocyte growth factor originating from pancreas cells may play a crucial role in facilitating pancreas cancer cell invasion and metastasis.

In multiple analysis by the multiple linear regression method, TNM staging ($p < 0.001$) seemed an independent factor regarding the significant higher serum levels of soluble hepatocyte growth factor. Based on the results, the higher level of serum hepatocyte growth factor is shown closely related to a more advanced TNM stage (Tab. 2). Thus, the level of serum hepatocyte growth factor may reflect the severity of invasive pancreatic cancer and can serve as a potential target for anticancerous drug design. It is worthwhile to have further investigation by larger group of patients with longer follow-up to achieve more substantial conclusion.

Reference

1. Parker SL, Tong T, Bolden S, Wingo PA. Cancer statistics. 1997 CA Cancer J Clin 1997; 47: 6—27.
2. Niederhuber JE, Brennan MF, Menck HR. The National Cancer Data Base report on pancreatic cancer. Cancer 1995; 76: 1671—1677.
3. Conlon KC, Klimstra DS, Brennan MF. Long-term survival after curative resection for pancreatic ductal adenocarcinoma. Clinicopathologic analysis of 5-year survivors. Ann Surg 1996; 223: 273—279.
4. Yamamoto S, Tomita Y, Hoshida Y et al. Prognostic significance of activated Akt expression in pancreatic ductal adenocarcinoma. Clin Cancer Res 2004; 10: 2846—2850.
5. Singh-Kaw P, Zarnegar R, Siegfried JM. Stimulatory effects of hepatocyte growth factor on normal and neoplastic human epithelial cells. Amer J Physiol 1995; 268: L1012—1020.
6. Rosen EM, Knesel J, Goldberg ID, Jin L et al. Scatter factor modulates the metastatic phenotype of the EMT6 mouse mammary tumor. Int J Cancer 1994; 57: 706—714.
7. Weidner KM, Behrens J, Vandekerckhove J et al. Scatter factor: molecular characteristics and effect on the invasiveness of epithelial cells. J Cell Biol 1990; 111: 2097—2108.
8. Montesano R, Matsumoto K, Nakamura T et al. Identification of a fibroblast-derived epithelial morphogen as hepatocyte growth factor. Cell 1991; 67: 901—908.
9. Grant DS, Kleinman HK, Goldberg ID et al. Scatter factor induces blood vessels formation in vivo. Proc Natl Acad Sci USA 1993; 90: 1937—1941.
10. Igawa T, Kanda S, Kanetake H et al. Hepatocyte growth factor is a potent mitogen for cultured rabbit renal tubular epithelial cells. Biochem Biophys Res Commun 1991; 174: 831—838.
11. Yamashita J, Ogawa M, Beppu T. Immunoreactive hepatocyte growth factor is present in tissue extracts from human breast cancer but not in conditioned medium of human breast cancer cell lines. Res Commun Chem Pathol Pharmacol 1993; 82: 249—252.
12. Siegfried JM, Weissfeld LA, Luketich JD et al. The clinical significance of hepatocyte growth factor for non-small cell lung cancer. Ann Thorac Surg 1998; 66: 1915—1918.
13. Sobin LH, Wittekind CH. TNM classification of malignant tumors. 6th ed. New York (NY): Wiley-Liss; 2002.
14. Shenn-Chen SM, Liu YW, Eng HL, Chou FF. Serum levels of Hepatocyte growth factor in patients with breast cancer. Cancer Epi Bio Prevent 2005; 14 (3): 715—717.
15. Jeffer M, Rong S, Anver M, Woude GFV. Autocrine hepatocyte growth factor/scatter factor-met signaling induces transformation and the invasive/metastatic phenotype in C127 cells. Oncogene 1996; 13: 853—861.
16. Bellusci S, Mones G, Gaudino G et al. Creation of an hepatocyte growth factor/scatter factor autocrine loop in carcinoma cells induces invasive properties associated with increased tumorigenicity. Oncogene 1994; 9: 1091—1099.
17. Seslar SP, Nakamura T, Stephen WB. Regulation of fibroblast hepatocyte growth factor/scatter factor expression by human breast carcinoma cell lines and peptide growth factors. Cancer Res 1993; 53: 1233—1238.
18. Tajima H, Matsumoto K, Nakamura T. Hepatocyte growth factor has potent anti-proliferative activity in various tumor cell lines. FEBS Lett 1991; 291: 229—232.
19. Matsumoto K, Tajima H, Nakamura T. Hepatocyte growth factor is a potent stimulator of human melanocyte DNA synthesis and growth. Biochem Biophys Res Commun 1991; 176: 45—51.

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