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Running title: c-Met overexpression in cholangiocarcinoma

Abstract

Background: Cholangiocarcinoma (CC) is a highly malignant carcinoma. We attempted to clarify the prognostic significance of c-Met overexpression and its association with clinicopathological factors in patients with cholangiocarcinoma (CC).

Patients and Methods: One hundred eleven patients with intrahepatic CC (IHCC) and 136 patients with extrahepatic CC (EHCC) who had undergone curative surgery were divided immunohistologically into c-Met^{high} and c-Met^{low} groups. Clinicopathological factors and outcomes were compared between the groups. c-Met and EGFR expression was also examined in ten CC cell lines.

Results: The positivity of c-Met was 45.0% in IHCC and 68.4% in EHCC; c-Met^{high} expression was demonstrated in 11.7% of IHCC and 16.2% of EHCC. c-Met^{high} expression was significantly correlated with the 5-year survival rate for CC overall (P=0.0046) and for IHCC (P=0.0013), histopathological classification in EHCC, and EGFR overexpression in both IHCC and EHCC. Co-expression and co-activation of c-Met and EGFR was also observed in CC cell lines. Multivariate analysis revealed that c-Met^{high} expression was an independent predictor of poor overall and disease-free survival in patients with IHCC.

Conclusions: c-Met overexpression is associated with EGFR expression and is a poor prognostic factor in CC.

Keywords: c-Met; cholangiocarcinoma; immunohistochemistry; prognostic factor; epidermal growth factor receptor

1. Introduction

Cholangiocarcinoma (CC) is a highly malignant invasive carcinoma arising through malignant transformation of cholangiocytes. Epidemiologic studies have demonstrated that the incidence and mortality rates of this disease, especially those of intrahepatic cholangiocarcinoma (IHCC), are increasing worldwide (Blechacz, Gores, 2008; Hezel, Zhu, 2008; Yachimski, Pratt, 2008; Mouzas et al, 2002; Okuda et al, 2002; Aljiffry et al, 2009).

It is difficult to diagnose CC at an early stage because of the lack of clinical symptoms at this point, and most patients have unresectable disease at clinical presentation. Surgical resection is the only curative therapy, but among those patients who receive it, recurrence rates are high (Hezel, Zhu, 2008). To date, no randomized study has demonstrated any clear survival benefit of a specific chemotherapeutic regimen for cases of unresectable and recurrent CC (Aljiffry et al, 2009). Existing phase II data and a more recent meta-analysis suggest that gemcitabine and gemcitabine-based platinum regimens offer a slight advantage over other regimens (Hezel, Zhu, 2008).

Recently, a new treatment strategy for cholangiocarcinoma has been proposed, in the light of better understanding of the molecular mechanisms of carcinogenesis: it has been proposed that receptor tyrosine kinases (RTKs), such as epidermal growth factor receptor (EGFR), vascular epithelial growth factor (VEGF) and c-Met, are promising targets for treatment of CC (Socoteanu et al, 2008; Yoshikawa et al, 2008). In a previous report, we have indicated that EGFR and VEGF could be promising molecules for targeted therapy of CC (Yoshikawa et al, 2008; Yoshikawa et al, 2009).

c-Met, also known as scatter factor, is a high-affinity receptor for hepatocyte growth factor (HGF). Activation of HGF-c-Met signaling initiates cell invasiveness and triggers metastasis through direct involvement of tumor angiogenesis (Zhang et al, 2003). Upon ligand binding, c-Met activates multiple downstream signal transduction pathways,

including the Grb2-Ras-mitogen-activated protein kinase (MAPK) cascade, the phosphatidylinositol-3 kinase (PI3K) pathway, and the signal transducer and activator of transcription (STAT) pathway (Furge et al, 2000; Weidner et al, 1993). C-Met partners include the integrin α6β4, CD44, plexin B, Fas and other RTKs such as RON, EGFR and ErbB2 (Gentile et al, 2008).

c-Met and EGFR are considered to assemble oncogenic signaling networks. Amplified c-Met activates members of the EGFR family and, conversely, mutated or amplified EGFR activates c-Met in vitro (Guo et al, 2008). EGFR is frequently coexpressed with c-Met in cell lines of lung, head and neck, breast, colon, and brain tumors (Reznik et al, 2008).

Enhanced expression of c-Met protein has been described in various solid tumors such as breast cancer (Garcia et al, 2007; Eder et al, 2009), esophageal adenocarcinoma (Herrera et al, 2005), gastric cancer (Ji et al, 2008; Drebber et al, 2008), colon cancer (Liu et al, 1992), lung cancer (Lutterbach et al, 2007; Nakamura et al, 2007), ovarian cancer (Sawada et al, 2007), brain tumor (Kong et al, 2009), hepatocellular carcinoma (Boix et al, 1994; Suzuki et al, 1994), and biliary tract carcinoma (Aishima et al, 2002; Terada et al, 1998; Hida et al, 1999; Nakazawa et al, 2005). Recently, it has been proposed that c-Met might be a promising target for treatment of CC (Socoteanu et al, 2008). However, no study has yet demonstrated its prognostic significance in CC.

To improve our understanding of the clinical significance of c-Met in CC, the primary aim of this study is to clarify the frequency of c-Met overexpression. Following with this analysis, the second aim of this study is to analyze its association with clinicopathological factors, along with molecular data (EGFR, HER2 and VEGF expression), in the largest cohort (111 cases of IHCC and 136 cases of extrahepatic cholangiocarcinoma (EHCC)) of surgical specimens of CC. We also examined the expression of c-Met and EGFR in cholangiocarcinoma cell lines.

2. Patients and methods

2.1. Patients

A total of 247 patients with CC were examined in the present study. The patients had undergone surgery and been diagnosed histologically as having adenocarcinoma of the bile duct, except for cancer of gallbladder and ampulla of Vater, at the National Cancer Center Hospital, Tokyo, between February 1990 and July 2005. Patients who had other malignancies or had died within four weeks after surgery were excluded. Clinical and pathological data were obtained from the medical records of the patients. To examine the correlations of c-Met with other RTKs (EGFR, HER2 or VEGF), qualified cases including previous data for overexpression of these molecules (Yoshikawa et al, 2008) were examined.

The studied patients included 168 men and 79 women ranging in age from 33 to 82 years (median 65 years), who had been observed for periods ranging from 1.4 to 204.5 months (median 29.8 months). The cases were divided into two groups, IHCC and EHCC, in accordance with the TNM Classification of Malignant Tumors (Sobin, Wittekind, 2010) defined by the International Union Against Cancer (UICC) and the World Health Organization Histological Classification of Tumors (Hamilton, Altonen, 2000). There were 111 cases of IHCC and 136 cases of EHCC. In this study, peri-hilar EHCC and distal EHCC are combined as EHCC because it is difficult to categorized EHCC based on the origin of the cystic duct. Tumor recurrence was defined as tumor growth in any site of the body after the operation, which was diagnosed by clinically, radiologically, or pathologically, but mainly by computed tomography and ultrasonography. Only tumor death was used for analysis. The research protocol was approved by the Ethics Committee of the National Cancer Center, Tokyo, Japan. All patients gave written informed consent for inclusion in this study.

2.2. Immunohistochemistry

Immunohistochemistry (IHC) was performed on 247 formalin-fixed, paraffin-embedded tissue sections. Immunohistochemical staining for c-Met was performed using a polymer-based method (Envision^{TM} + Dual link-system-HRP (Dako, DK-2600 Glostrup, Denmark)). Serial sections (4 μm thick) cut from representative paraffin-embedded serial tissue slices were prepared on silicone-coated slides for IHC evaluation. Sections cut through the maximum tumor diameter were selected for IHC evaluation. The sections were deparaffinized in xylene, and rehydrated through graded concentrations of ethanol (50-100%). Endogenous peroxidase activity was blocked by incubation in 0.3% hydrogen peroxide solution for 30 min. The antigens were retrieved by heating in a pressure cooker at 121 °C for 10 min in 0.01M citrate buffer. The tissue sections were incubated overnight at 4°C with anti-c-Met primary antibody (rabbit polyclonal; IBL, Gunma, Japan) at a dilution of 1:50. After a washing in PBS, the sections were treated with Envison+ Dual link reagent at room temperature for 30 min. 3,3'-Diaminobenzidine tetrahydrochloride was used as the chromogen, and the tissue sections were counterstained with hematoxylin.

Intensities of c-Met immunoreactivity were defined as: 0, complete absence of membrane staining or membrane staining in less than 30% of cancer cells; 1+, faint and partial membrane staining in at least 30% of cancer cells; 2+, strong and complete staining in at least 30% of cancer cells. The cases were divided into two groups, c-Met^{low} (0 or 1+) or c-Met^{high} (2+), for purposes of statistical analysis. The sections were evaluated by three observers, M.M., H.O., and T.S. without knowledge of the clinical data. H.O. and T.S. are board-certified pathologists. IHC of EGFR and assessment of its expression were done as described previously (Yoshikawa et al, 2008).

2.3. Cell lines

NCC-CC1, NCC-CC3-1, NCC-CC3-2, and NCC-CC4 cells were established from human IHCC, and NCC-BD1and NCC-BD2 from human EHCC, at the National Cancer Center Research Institute (Ojima et al, 2010). TKKK, HuCCT1, OZ, TGBC24TKB, and MKN45 were purchased from RIKEN Bio Resource Center or from the Japanese Collection of Research Bioresources. TKKK, TGBC24TKB and HuCCT1 were established from IHCC, and OZ was from EHCC. MKN45 was a gastric cancer cell line which was used as a positive control, because of its high expression of c-Met and phospho-Met (Smolen et al, 2006). All of the cell lines had been derived from Japanese patients. The originally established six CC cell lines, HuCCT1 and MKN45 were maintained in RPMI with 10% bovine serum. TGBC24TKB, TKKK, and OZ were maintained in Dulbecco's modified Eagle medium with 10% bovine serum.

2.4. Western blotting

Subconfluent cells were lysed at 4 °C for 30 min using lysis buffer containing 10 mM Tris-HCl (pH 7.5), 1% Triton X-100, and 150 mM NaCl with a complete protease inhibitor cocktail (Roche) and a phosphate inhibitor cocktail (Nacalai Tesque, Kyoto, Japan). The protein concentration was determined using a Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA). Lysates (5 µg protein/well) were separated by SDS-PAGE, then transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). The membranes were blocked with 5% skim milk in PBS for 30 min and then probed with the following primary antibodies: anti-c-Met (rabbit polyclonal; IBL, Gunma, Japan; 1:1000), anti-phospho-Met (pY1234/1235, Rabbit monoclonal, clone D26; Cell Signaling Technology, Danvers, MA; 1:1000), anti-EGFR (mouse monoclonal, clone 31G7; Zymed, South San Francisco, CA, USA; 1:1000) and anti-phospho EGFR (pY1173, rabbit monoclonal, clone 53A5; Cell Signaling Technology) at 4 °C overnight. After washing with PBS-Tween 20 (0.5 %), the

membranes were re-blocked and then incubated at room temperature for 1 h with horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit antibody at a dilution of 1:1000. Following three washes, bands were visualized using the ECL Western Blotting Detection Reagents (GE Healthcare UK Ltd., Buckinghamshire, England). Anti-β-actin (mouse monoclonal; clone AC-15, Sigma, St Louis, MO, USA) was used as a loading control.

2.5. Statistics

Correlations between the results of immunohistochemistry and clinicopathological factors were determined by Fisher's exact probability test, except for histopathological classification, which was analyzed by chi-squared test. Cumulative survival rates and survival curves were calculated by the Kaplan–Meier method, and log-rank test was performed for the comparison of survival curves between low and high groups defied by c-Met expression level. The Cox proportional hazards model was used to estimate the hazard ratio and 95% confidence interval of each outcome (tumor death and recurrence). Multivariate analysis was performed for factors selected as risk factors by univariate analysis, except for UICC pT and UICC Stage, which are composed of other factors. Correlations between the intensity of c-Met and that of EGFR in immunohistochemistry or Western blotting were determined by Spearman's rank correlation. Statistical analysis was done using the Statview 5.0 statistical software package (Abacus Concepts, Berkeley, CA, USA). The level of significance was set at $p<0.05$.

3. Results

3.1. Immunohistochemical analysis of c-Met in human cholangiocarcinoma specimens

c-Met staining was localized in both the cell membrane and cytoplasm of CC cells (Figure 1). Strong immunostaining for c-Met was apparent at the luminal cell surface of neoplastic glands and ducts of adenocarcinoma. Positive staining for c-Met was demonstrated in 143 (57.9%, 95%CI: 51.7-64.1) of the 247 cases of CC overall, 50 (45.0%, 95%CI: 35.7-54.3) of the 111 cases of IHCC, and 93 (68.4%, 95%CI: 60.6-76.2) of the 136 cases of EHCC; High c-Met expression (2+) was demonstrated in 35 (14.2%, 95%CI: 9.8-18.6) of the 247 cases of CC overall, 13 (11.7%, 95%CI: 5.7-17.7) of the 111 cases of IHCC, and 22 (16.2%, 95%CI: 10.0-22.4) of the 136 cases of EHCC. When compared with EGFR staining, we occasionally observed co-expression of c-Met and EGFR (Figure 2).

3.2. c-Met and EGFR expression in CC cell lines

Expression of c-Met, phospho-Met, EGFR, and phospho-EGFR in ten CC cells and one gastric cancer cells were estimated by Western blotting (Figure 3). Expression of c-Met was observed in nine CC cells. Co-expression of c-Met and EGFR was detected in eight of them (except NCC-CC3-1). Prominent c-Met phosphorylation was detected in five cell lines (HuCCT1, OZ, NCC-BD2, TGBC24TKB and NCC-BD1) and simultaneous activation of c-Met and EGFR was observed in seven cell lines including these five.

3.3. Correlations between c-Met and clinicopathological factors

The relationships between c-Met expression and clinicopathological factors of IHCC and EHCC were evaluated and are shown in Tables 1 and 2. Increased expression of c-Met was significantly correlated with overexpression of EGFR in IHCC (P=0.0063),

and histopathological classification (P=0.0239) and overexpression of EGFR (P=0.0056) in EHCC. No other clinical factors were associated with c-Met expression.

Five-year survival for patients in the c-Met^{high} and c-Met^{low} groups was 15.4% and 41.1% (p=0.0013) for IHCC and 40.9% and 45.8% (P=0.1396) for EHCC, respectively (Figure 4). We then performed multivariate analysis to assess the prognostic significance of c-Met expression. In IHCC, the independent predictors of poor overall survival were high c-Met expression (HR:3.92, 95%CI:1.62-9.48), macroscopic type (HR:4.57, 95%CI:1.44-14.51), intrahepatic metastasis (HR:3.27, 95%CI:1.78-5.99) and lymph node metastasis (HR:1.99, 95%CI:1.11-3.59). High c-Met expression (HR:3.50, 95%CI:1.56-7.85), macroscopic type (HR:4.78, 95%CI:1.69-13.4), intrahepatic metastasis (HR:2.78, 95%CI:1.60-4.82), lymph node metastasis (HR:2.94, 95%CI:1.70-5.08), venous invasion (HR:4.62, 95%CI:1.13-18.8) and EGFR overexpression (HR:1.98, 95%CI:1.12-3.51) were significant predictors of disease-free survival (Table 3).

In EHCC, the c-Met^{high} group tended to have a poor 5-year survival rate, but not to a significant degree. Univariate analysis also showed that c-Met^{high} was not a significant factor for survival. Therefore, multivariate analysis was not performed for EHCC.

4. Discussion

In the present study, we have demonstrated the importance of c-Met overexpression in the prognosis and treatment of CC. We found that c-Met expression was correlated with EGFR overexpression in CC, and that it was also a significant prognostic factor in IHCC. In previous studies, the frequency of c-Met overexpression ranged from 21% to 58% in IHCC (Aishima et al, 2002; Terada et al, 1998; Nakazawa et al, 2005) and from 0% to 80% in EHCC (Hida et al, 1999; Nakazawa et al, 2005). This rather broad range is probably attributable to the small numbers of cases studied, or to differences in the definition of positivity. Moreover, no correlation between c-Met overexpression and clinical outcome of CC has been demonstrated previously. Here we showed that increased expression of c-Met was significantly associated with decreased overall and disease-free survival in patients with IHCC. The reason why c-Met expression was not a prognostic factor in EHCC may be partly explained by variables associated with their anatomic behavior and methods of surgery.

Simultaneous expression of c-Met and EGFR has been observed in clinical specimens of primary chordoma (Weinberger et al, 2005) and gastrinoma (Peghini et al, 2002). Accumulated evidence has suggested that cross-talk occurs between c-Met and EGFR in several cancer cell lines (Guo et al, 2008; Farazi et al, 2006; Jo et al, 2000). Here we showed that c-Met expression was correlated with EGFR expression in clinical specimen of CC. We found that both EGFR and c-Met are broadly activated in CC cell lines. Eight CC cells co-expressed both c-Met and EGFR and co-activation of both proteins was detected in seven CC cell lines. It has been proposed that amplified c-Met drives the activity of EGFR family members and that mutated and amplified EGFR can drive c-Met activity (Guo et al, 2008). Mutual or unidirectional interaction between EGFR and MET activation has been reported in several cell lines (Reznik et al, 2008; Jo et al, 2000; Bergstrom et al, 2000). It is thought that either c-Met or EGFR stands at the

top of the hierarchy of the downstream signaling pathway governed by the two molecules in a subset of cancer.

Collectively, it seems reasonable that efficient molecular therapy for CC should target multiple kinases such as c-Met, EGFR and VEGFR. c-Met activation is regarded as one of the molecular mechanisms involved in the acquisition of resistance to anti-EGFR therapy, since activation of the alternative RTK pathway would bypass the EGFR pathway (Dempke, Heinemann, 2009). Therefore, inhibition of c-Met, either alone or in combination with an EGFR inhibitor, may be clinically beneficial in the setting of EGFR inhibitor resistance (Eder et al, 2009). Several studies have focused on combination therapy with c-Met inhibitors and agents targeting EGFR family members (Toschi, Janne, 2008).

In conclusion, c-Met overexpression is significantly correlated with overexpression of EGFR in CC and with prognosis in IHCC. Further molecular investigation of the interaction between EGFR and c-Met in this fatal disease is urgently needed.

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Figure Legends

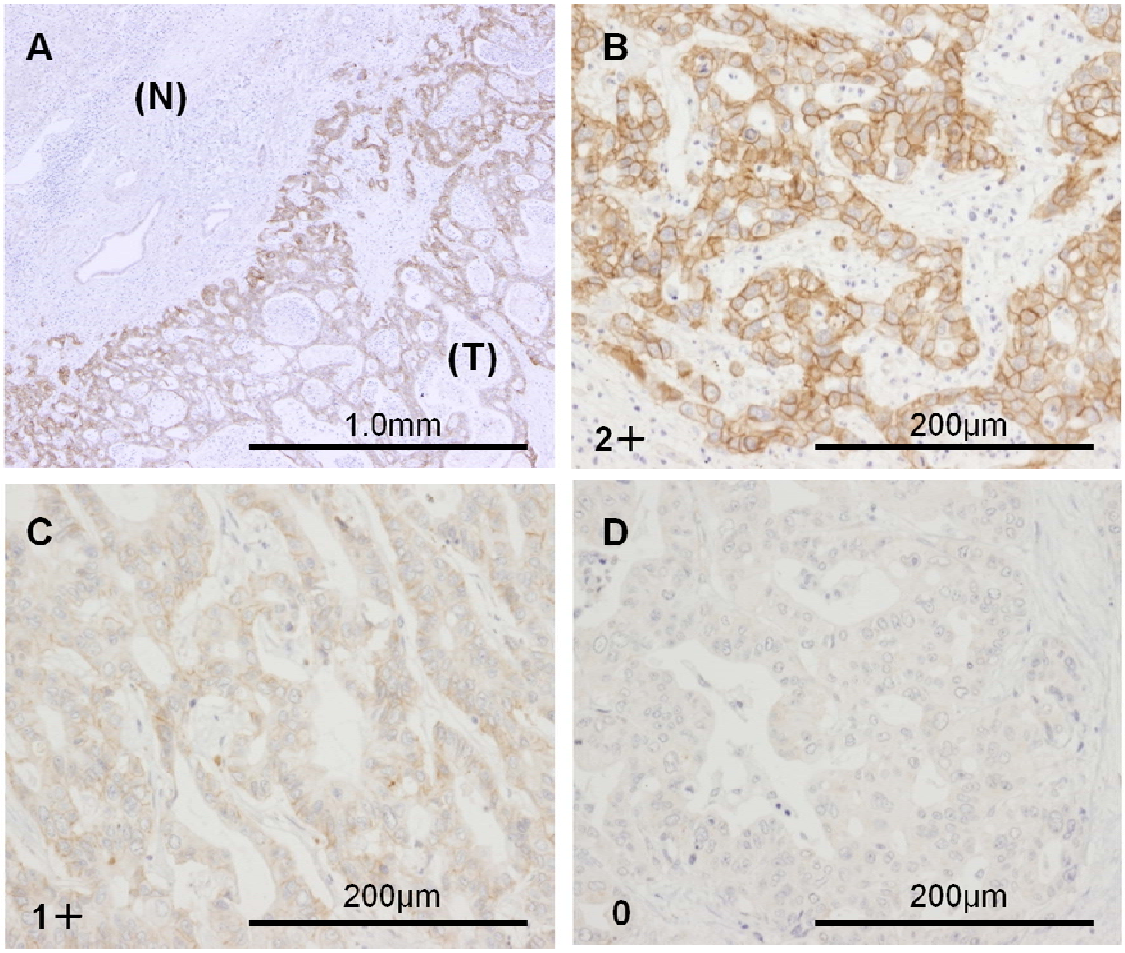

Figure 1

c-Met expression in primary cholangiocarcinoma cases. (A) c-MET expression was exclusively detected in tumor cells (T), but not in non-cancerous bile duct epithelium (N). (B-D) Representative immunohistochemistry pictures of higher magnification of c-Met expression (expression score is 2+(B), 1+ (C) and 0(D), respectively). c-MET is localized in both the cell membrane and cytoplasm of CC cells. Scale bar indicates 1.0mm (A) and 200μm (B-D).

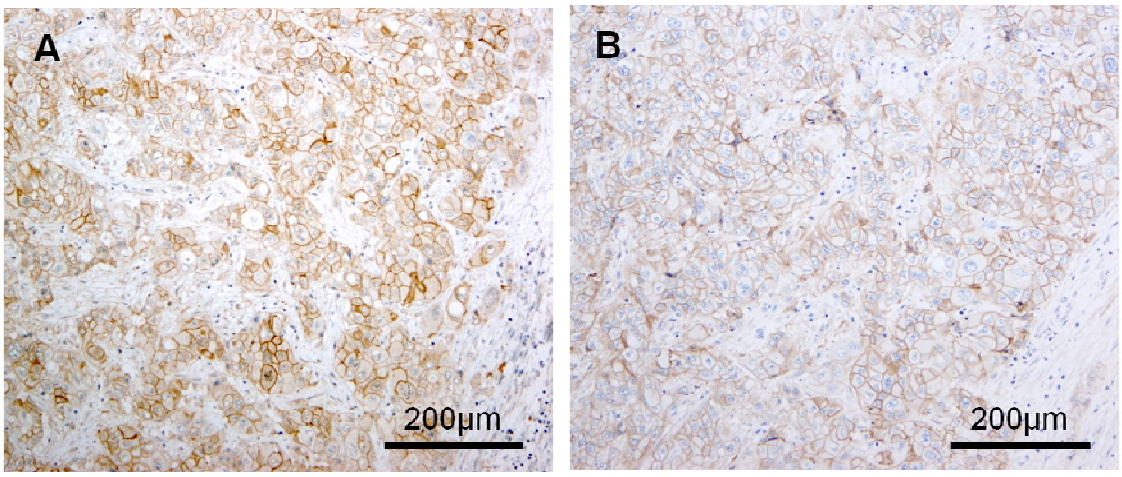

Figure 2

A representative case showing co-expression of c-Met (A) and EGFR (B) in adjacent sections of the same tumor. Scale bar indicates 200μm.

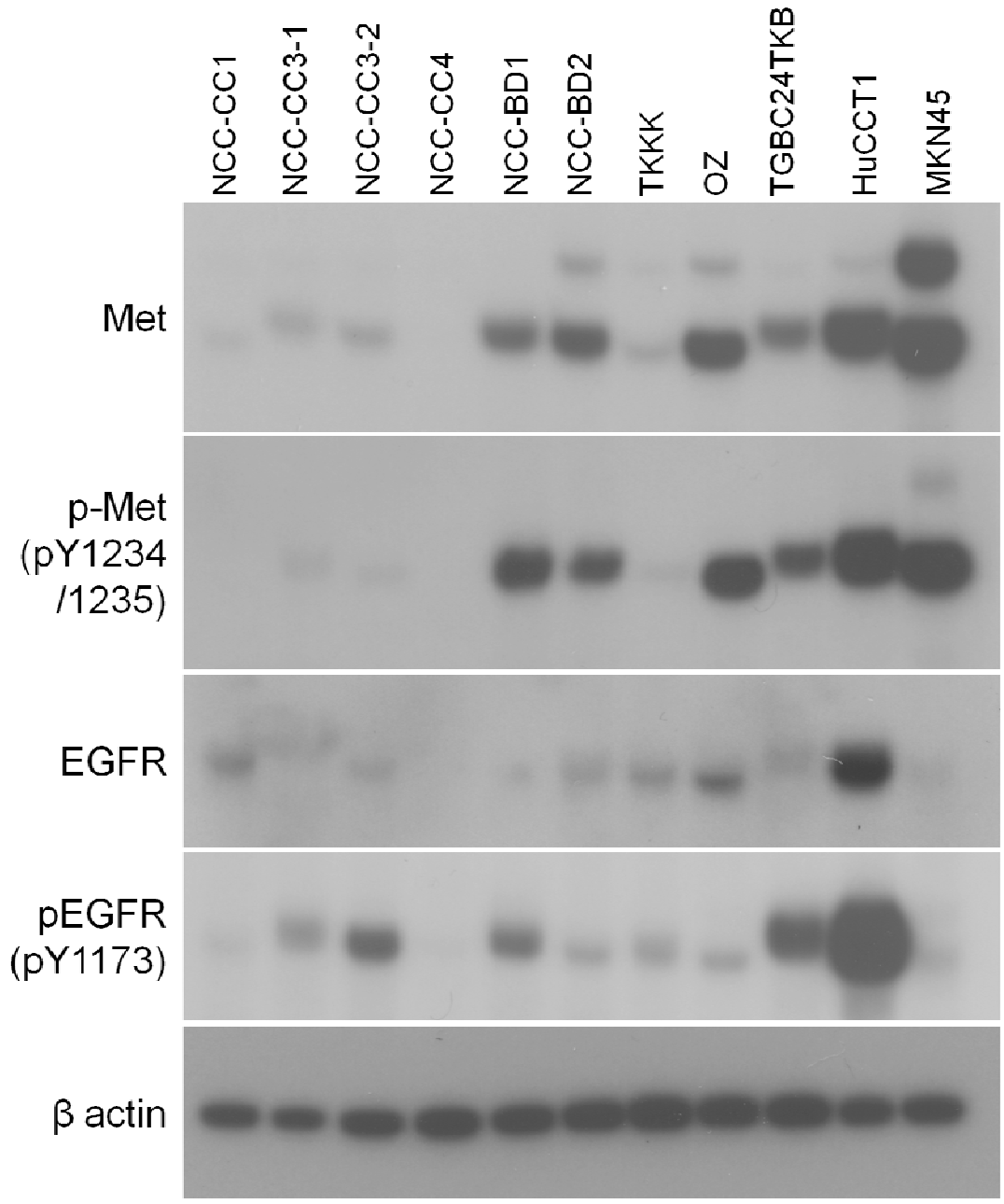

Figure 3

Immunoblot analysis of c-Met, phosphorylated-Met pY1234/1235), EGFR and phosphorylated EGFR (pY1173) in cholangiocarcinoma cell lines. MKN45 cell (a human gastric cancer cell) is a positive control of c-Met and phosphorylated-Met expression (Smolen at al, 2006). β-actin is a loading control.

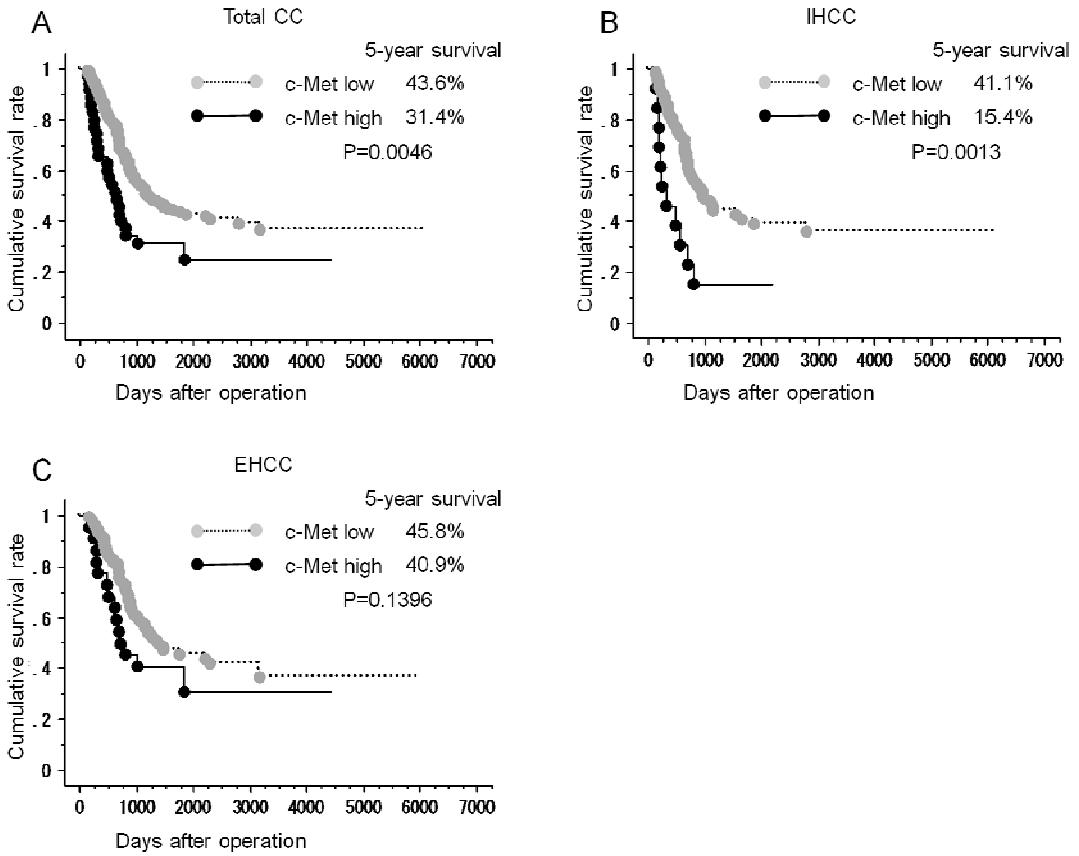

Figure 4

Survival curves according to c-Met expression. High c-Met expression was significantly correlated with poor survival in patients with cholangiocarcinoma (CC) as a whole (**A**) and in those with intrahepatic CC (IHCC) (**B**), but not in those with extrahepatic CC (EHCC) (**C**).

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Table1.
Comparison of clinicopathological factors between patients with high and low c-Met expression in IHCC.

		c-Met high	c-Met low	p-value
Gender				
	Male	7	59	0.7636
	Female	6	39	
Age				
	65 or over	9	48	0.2396
	Under 65	4	50	
Tumor size				
	5cm or over	8	39	0.2430
	Under 5cm	5	52	
Macroscopic type				
	Mass forming	10	83	0.4397
	Non-mass forming	3	15	
Intrahepatic metastasis				
	Negative	8	70	0.5229
	Positive	5	28	
Invasion to hepatic vein				
	Negative	5	53	0.2496
	Positive	8	41	
Invasion to portal vein				
	Negative	1	24	0.2907
	Positive	12	73	
Lymph node metastasis		7		
	Negative	6	57	0.7739
	Positive		41	
Histopathological classification				
	Well differentiated	4	21	0.5943
	Moderately differentiated	8	73	
	Poorly differentiated	1	4	
UICC pT				
	is+1+2a+2b	4	28	>0.9999
	3+4	9	70	
UICC Stage				
	I+II	8	51	0.5680
	III+IVA	5	47	
Lymphatic vessel invasion				
	Negative	2	37	>0.9999
	Positive	11	61	
Venous invasion				
	Negative	1	19	0.4566
	Positive	12	79	
Perineural invasion				
	Negative	4	27	0.7536
	Positive	9	71	
Hepatic surgical margin				
	Negative	9	84	0.2202
	Positive	4	14	
Bile duct margin				
	Negative	10	86	0.3797
	Positive	3	12	
EGFR expression				
	Negative	5	72	0.0063
	Positive	8	21	
VEGF expression				
	Negative	7	51	0.5697
	Positive	6	42	
HER2 expression				
	Negative	13	92	>0.9999
	Positive	0	1	

Table 2.
Comparison of clinicopathological factors between patients with high and low c-Met expression in EHCC.

		c-Met		p-value
		high	low	
Gender				
	Male	16	86	0.7914
	Female	6	28	
Age				
	65 or over	16	59	0.1004
	Under 65	6	55	
Tumor size				
	3cm or over	11	63	0.8144
	Under 3cm	10	50	
Macroscopic type				
	Polypoid	3	19	>0.9999
	Non-polypoid	18	91	
Depth of tumor invasion				
	within fm	2	13	>0.9999
	beyond fm	20	101	
Invasion to hepatic artery				
	Negative	21	111	0.5106
	Positive	1	3	
Invasion to portal vein				
	Negative	20	82	0.0649
	Positive	2	32	
Lymph node metastasis				
	Negative	10	65	0.3554
	Positive	12	49	
Histopathological classification				
	Papillary	4	18	0.0239
	Well differentiated	2	30	
	Moderately differentiated	9	55	
	Poorly differentiated	7	11	
Lymphatic vessel invasion				
	Negative	2	98	0.7369
	Positive	20	16	
Venous invasion				
	Negative	3	18	>0.9999
	Positive	19	96	
Perineural invasion				
	Negative	4	21	>0.9999
	Positive	18	93	
Dissected periductal structures margin				
	Negative	18	97	0.7480
	Positive	4	17	
Bile duct margin				
	Negative	16	82	>0.9999
	Positive	6	32	
Invasion to other organ				
	Negative	12	44	0.2363
	Positive	10	70	
EGFR expression				
	Negative	12	93	0.0056
	Positive	9	16	
VEGF expression				
	Negative	7	46	0.4798
	Positive	14	63	
HER2 expression				
	Negative	19	100	>0.9999
	Positive	2	9	

Table 3.
Multivariate analyses of overall survival and disease-free survival in patients with IHCC. (Cox proportional hazards model)

	Overall survival			Disease-free survival		
	HR	95% CI	p-value	HR	95% CI	p-value
Macroscopic type						
Mass forming	4.572	1.440-14.516	0.0099	4.783	1.698-13.470	0.0030
Non-mass forming	1.00			1.00		
Intrahepatic metastasis						
Negative	1.00			1.00		
Positive	3.270	1.783-5.999	0.0001	2.781	1.604-4.822	0.0003
Invasion to portal vein						
Negative	1.00			-		
Positive	0.881	0.388-1.999	0.7623	-	-	-
Lymph node metastasis						
Negative	1.00			1.00		
Positive	1.998	1.110-3.597	0.0209	2.947	1.707-5.088	0.0001
Histopathological classification						
Well differentiated	1.00			1.00		
Moderately differentiated	1.507	0.639-3.554	0.3491	0.753	0.345-1.642	0.4759
Poorly differentiated	2.031	0.526-7.835	0.3036	1.199	0.340-4.227	0.7772
Lymphatic vessel invasion						
Negative	1.00			1.00		
Positive	3.119	0.851-11.435	0.0860	2.723	0.759-9.768	0.1243
Venous invasion						
Negative	1.00			1.00		
Positive	3.121	0.825-11.807	0.0935	4.628	1.136-18.854	0.325
Perineural invasion						
Negative	1.00			1.00		
Positive	0.588	0.265-1.305	0.1917	0.511	0.244-1.072	0.756
Bile duct margin						
Negative	1.00			-		
Positive	1.871	0.902-3.882	0.0926	-	-	-
EGFR expression						
Negative	1.00			1.00		
Positive	1.745	0.957-3.180	0.0690	1.987	1.125-3.511	0.0180
c-Met expression						
Negative	1.00			1.00		
Positive	3.921	1.620-9.487	0.0003	3.502	1.562-7.851	0.0023

Abbreviations: HR = Hazard ratio, CI = Confidence interval