Expression of insulin-like growth factor I receptor as a biomarker for predicting prognosis in biliary tract cancer patients

HIDEO SUZUKI[1], JUAN C. ROA[2], TORU KAWAMOTO[3], KAZUNORI ISHIGE[1],
IGNACIO I. WISTUBA[4], DONGHUI LI[5], MELANIE B. THOMAS[6] and JUNICHI SHODA[1]

[1]Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki 305-8575, Japan; [2]Molecular Pathology Laboratory,
Department of Pathology, Pontifical Catholic University of Chile, Santiago 8320000, Chile; [3]Minato Shiba Clinic,
Tokyo 105-0014, Japan; Departments of [4]Pathology and [5]Gastrointestinal Medical Oncology, The University of Texas
MD Anderson Cancer Center, Houston, TX 77030; [6]Medical University of South Carolina,
Hollings Cancer Center, Charleston, SC 29425, USA

Abstract. Carcinomas of the gallbladder (GBCa) and bile ducts are aggressive tumors with poor survival and it is, therefore, essential to elucidate the molecular mechanisms of the various signaling pathways in order to develop effective therapies. In this study, tumor specimens from 40 GBCa patients, 12 extrahepatic bile duct carcinoma patients and 26 intrahepatic bile duct carcinoma patients from the USA and Japan were investigated for insulin-like growth factor I receptor (IGF-IR), mammalian target of rapamycin (mTOR) and rapidly accelerated fibrosarcoma-1 (Raf-1) expression by immunohistochemistry; in addition, the correlations with histological type, pathological stage and patient outcome were analyzed. Positive expression of IGF-IR, mTOR and Raf-1 were identified in 68, 73 and 85% of the specimens, respectively. There was no association with histological type and pathological stage, although the positive expression rate of Raf-1 was higher in advanced-stage GBCa. Moreover, patients with positive expression of IGF-IR exhibited significantly reduced survival compared to those with negative IGF-IR expression. In conclusion, IGF-IR, mTOR and Raf-1 were highly expressed in biliary tract cancer and targeted therapy against IGF-IR may be an effective strategy. Among these molecules, IGF-IR expression was found to be a useful biomarker for identifying patients who may benefit from additional treatment.

Correspondence to: Dr Junichi Shoda, Faculty of Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8575, Japan
E-mail: shodaj@md.tsukuba.ac.jp

Introduction

Cancers of the gallbladder (GBCa) and biliary tract are highly lethal, as they are usually detected at an advanced stage and effective chemotherapy agents have not yet been developed. The 5-year survival rate for patients with advanced biliary tract cancer (BTC) is <5% (1). These aggressive malignancies are uncommon in the USA, accounting for an estimated 7,480 new cases and 3,340 deaths in 2005 (2,3); however, they are endemic in India, Pakistan, Korea, Japan, Eastern Europe and certain South American countries. In Chile, GBCa is the leading cause of cancer-related mortality in women (4-6).

One of the major clinical issues in BTC treatment is the identification of prognostic factors that affect patient survival in order to establish effective treatment strategies. It would be beneficial if clinicians were able to predict, from surgical specimens obtained at initial surgery, the malignant potential and post-surgical survival in order to determine the role for adjuvant therapy. However, the commonly used indicators of prognosis, including pathological stage and histological grade, do not adequately predict the clinical course of the majority of biliary malignancies or the biological characteristics of specific tumors. Therefore, novel prognostic biomarkers are required for predicting the aggressiveness of BTC.

The details of tumorigenesis, growth and progression of this disease are complex and have not been fully elucidated. Certain predisposing factors, such as chronic cholecystitis, cholelithiasis, obesity and the presence of an anomalous pancreaticobiliary junction have been associated with the risk of this disease (7). Several signaling pathways have been shown to be involved in the carcinogenesis of BTC. We previously reported that positive expression of epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2) was detected in 11.7 and 31.6% of BTC cases, respectively, indicating the possibility of anti-HER2 therapies against BTC (8). Genetic alterations in KRAS may also contribute to the development of certain types of GBCa (9). However, the published data vary and there have been no systematic studies to evaluate other proteins that may be useful biomarkers.

Table I. Characteristics of patients with biliary tract cancer.

Characteristics	GBCa (n=40)	EHBDCa (n=12)	IHBDCa (n=26)
Sources			
Tsukuba University Hospital	33	7	15
MDACC	7	5	11
Gender			
Male/female	16/24	6/6	19/7
Mean age, years (range)	64 (36-89)	61 (34-84)	62 (38-77)
Histological grade			
Well differentiated adenocarcinoma	17	2	4
Moderately differentiated adenocarcinoma	16	8	16
Poorly differentiated adenocarcinoma	3	1	5
Unclassified adenocarcinoma	4	1	1
pStage (AJCC, 6th edition)			
I	10	2	2
II	6	2	5
III	2	2	10
IV	22	5	8
Unknown	0	1	1

GBCa, gallbladder carcinoma; EHBDCa, extrahepatic bile duct carcinoma; IHBDCa, intrahepatic bile duct carcinoma; MDACC, MD Anderson Cancer Center; AJCC, American Joint Committee on Cancer.

In this study, we focused on insulin-like growth factor type I receptor (IGF-IR), mammalian target of rapamycin (mTOR) and rapidly accelerated fibrosarcoma-1 (Raf-1), as the roles of these proteins in BTC have not been fully elucidated. IGF-IR is a cell membrane receptor that participates in cell proliferation, differentiation and prevention of apoptosis (10). IGF-IR has been reported to be associated with clinical outcome in breast and gastric cancer (11,12). The mTOR and Raf-1 genes are also involved in the regulation of cell growth and proliferation in carcinogenesis. In the present study, the immunohistochemical expression of these proteins was investigated in formalin-fixed, paraffin-embedded surgical specimens from patients with BTC at different pathological stages and the clinical outcomes were compared. This study was based on the knowledge that the IGF-IR family of onco-genes is important in several tumor types and that several therapeutic agents targeting this gene family are clinically available. In order to establish a patient selection strategy for targeted therapy and to identify a useful predictive marker in BTC for improving the therapeutic options for these patients, we analyzed the baseline expression profiles of these gene targets in a large number of patient tumor specimens using immunohistochemistry (IHC).

Patients and methods

Tissue specimens. A total of 40 patients with GBCa, 12 with extrahepatic bile duct carcinoma (EHBDCa) and 26 with intrahepatic bile duct carcinoma (IHBDCa) from Tsukuba University Hospital in Japan and the University of Texas MD Anderson Cancer Center in the United States undergoing surgical tumor resection between 1991 and 2006 were selected for this study. The research protocol was approved by the Institutional Review Board of Tsukuba University Hospital. Prior to surgery, written informed consent was obtained from all the patients regarding use of their residual tissue for future research, including this study.

The mean age of the patients was 63 years (range, 34-89 years) and the patients included 41 men and 37 women. The demographic characteristics, pathological staging and histological findings (grade and type) according to the American Joint Committee on Cancer (AJCC) criteria are summarized in Table I. The surgically resected tumor specimens were fixed in 10% buffered formalin for 24 h, embedded in paraffin and 4-μm sections were obtained. The tissue sections were placed on silane-coated slides and used for hematoxylin and eosin (H&E) staining and detection of IGF-IR, mTOR and Raf-1 by IHC.

Procedures of immunohistochemical staining. Routine H&E staining was performed for morphological investigation. Immunostaining for IGF-IR, mTOR and Raf-1 using a Dako EnVision™+ dual link kit (Dako A/S, Glostrup, Denmark) was performed according to the manufacturer's instructions. Briefly, following deparaffinization and rehydration, the sections were brought to the boil in 10 mmol/l sodium citrate buffer (pH 6.0) at high power by microwave treatment for 10 min. Endogenous peroxidase activity was blocked by incubation with Dual Endogenous Enzyme Block (Dako A/S) for 10 min. After washing with phosphate-buffered saline
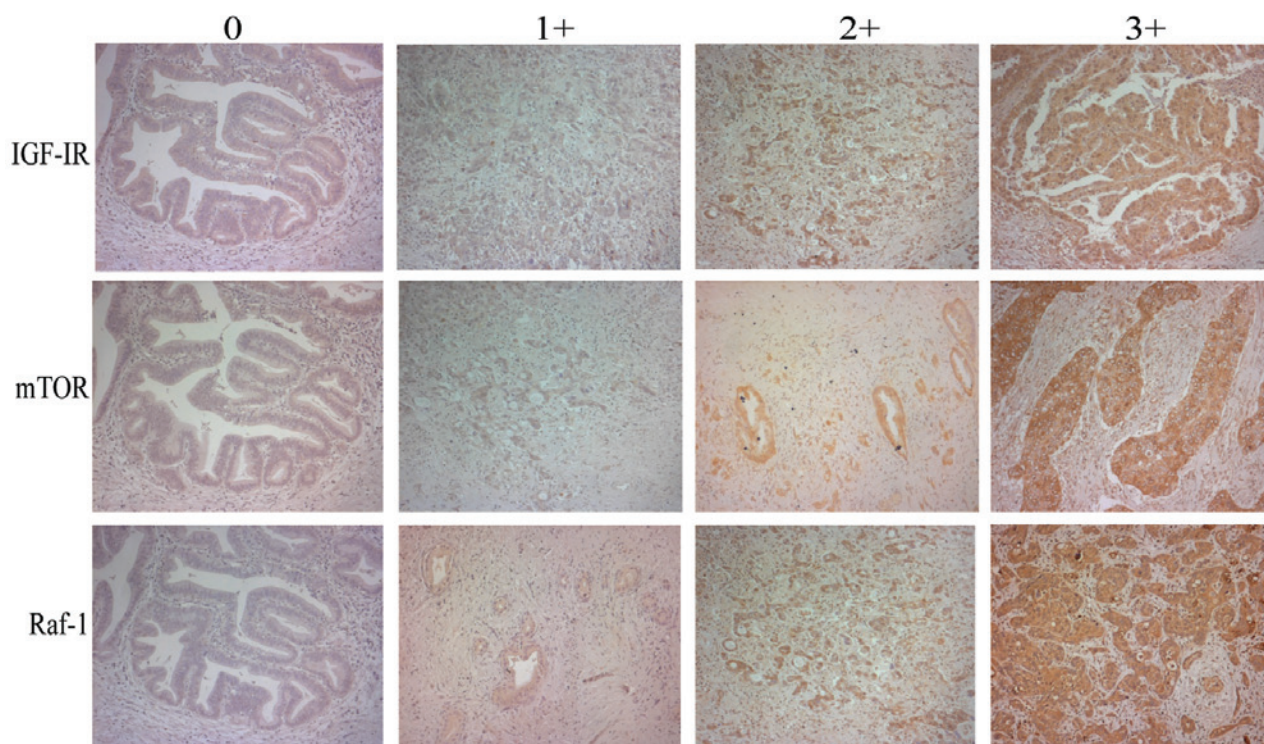
Figure 1. Representative photomicrographs showing typical immunohistochemical staining. 0, no staining; 1+, faint staining; 2+, weak to moderate staining; 3+, strong staining. Original magnification, x40. IGF-IR, insulin-like growth factor type I receptor; mTOR, mammalian target of rapamycin; Raf-1, rapidly accelerated fibrosarcoma-1.

(PBS, pH 7.4), the sections were incubated in blocking solution for 30 min to block non-specific staining. The following antibodies were used: Rabbit anti-IGF-IR (Cat. no. sc-713; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), rabbit anti-mTOR (Cat. no. NB100-240; Novus Biologicals, Littleton, CO, USA) and mouse anti-Raf-1 (Cat. no. sc-713; Santa Cruz Biotechnology). The sections were incubated overnight at 4°C with the respective primary antibodies at the optimized dilutions of 1:200 for IGF-IR, 1:100 for mTOR and 1:50 for Raf-1. After washing in PBS, the sections were incubated at room temperature with labeled polymer-horseradish peroxidase (Dako A/S) for 60 min and washed in PBS, followed by treatment with diaminobenzidine (KPL, Inc., Gaithersburg, MD, USA) solution for 30 sec. After stopping the reaction with PBS, the sections were counterstained with hematoxylin.

Evaluation of immunohistochemical staining. The evaluation of the sections was performed by two independent observers (H.S. and J.C.R) with respect to the histopathological characteristics and specific immunoreactivity. For the scoring of each protein expression, the membrane staining intensity and pattern were scored as follows: 0, no staining; 1+, faint staining; 2+, weak to moderate staining; and 3+, strong staining, with reference to the HercepTest scoring system. Each staining was interpreted as negative (0 or 1+) or positive (2+ or 3+) for each protein expression. Representative immunohistochemical staining of each antibody is shown in Fig. 1.

Statistical analysis. A two-sided χ^2 test or the Fisher's exact test was used for comparison of immunohistochemical data between groups. Survival curves were constructed using the

Table II. Positive expression rates of IGF-IR, mTOR and Raf-1 in biliary tract cancer (BTC).

BTC	IGF-IR, n/total (%)	mTOR, n/total (%)	Raf-1, n/total (%)
GBCa	30/40 (75)	28/40 (70)	34/40 (85)
EHBDCa	9/12 (75)	8/12 (67)	9/12 (75)
IHBDCa	14/26 (54)	21/26 (81)	23/26 (88)
Total	53/78 (68)	57/78 (73)	66/78 (85)

IGF-IR, insulin-like growth factor type I receptor; mTOR, mammalian target of rapamycin; Raf-1, rapidly accelerated fibrosarcoma-1; GBCa, gallbladder carcinoma; EHBDCa, extrahepatic bile duct carcinoma; IHBDCa, intrahepatic bile duct carcinoma.

Kaplan-Meier method and differences among survival curves were compared using the log-rank test. All the statistical analyses were performed using Statcel software (OMS Publishing, Saitama, Japan). P<0.05 was considered to indicate a statistically significant difference.

Results

IGF-IR, mTOR and Raf-1 IHC in BTC. The results of the analysis of the expression of IGF-IR, mTOR and Raf-1 in carcinomas arising in different sites of the biliary tract are summarized in Table II. Specimens from a total of 78 BTC patients were used for immunohistochemical analysis. The positive staining rate was ~70-80%, except for IGF-IR in IHBDCa (54%). However,

Table III. Immunohistochemical positive expression rates according to histological differentiation and pathological stage.

Variables	IGF-IR, n/total (%)	P-value	mTOR, n/total (%)	P-value	Raf-1, n/total (%)	P-value
All (n=78[a])						
Histological grade		0.13		0.28		0.47
Well/moderate	44/63 (70)		46/63 (73)		53/63 (84)	
Poor	4/9 (44)		8/9 (89)		7/9 (78)	
Pathological stage		0.72		0.46		0.96
I	9/14 (64)		11/14 (79)		10/14 (71)	
II-IV	42/62 (68)		45/62 (73)		54/62 (87)	
GBCa (n=40)						
Pathological stage		0.95		0.36		0.03
I	6/10 (60)		8/10 (80)		6/10 (60)	
II-IV	24/30 (80)		20/30 (67)		28/30 (93)	

The Fisher's exact test was used for comparison between groups. [a]A total of 72 samples was analysed in histological grade. IGF-IR, insulin-like growth factor type I receptor; mTOR, mammalian target of rapamycin; Raf-1, rapidly accelerated fibrosarcoma-1; BTC, biliary tract cancer; GBCa, gallbladder carcinoma.

the differences in the positive expression rate between cancer sites was not statistically significant.

Association of positive expression of IGF-IR, mTOR and Raf-1 with histological grade of BTC. The frequency of protein expression according to the AJCC histological grade classification is shown in Table III. For statistical analysis, the specimens were grouped as AJCC histological grades of well/moderately differentiated carcinoma vs. poorly differentiated carcinoma. The results demonstrated that there was no association between histological grade and the positive expression of these proteins. Moreover, no statistical significance was found in a subanalysis for IHBDCa, EHBDCa and GBCa (data not shown).

Association of positive expression of IGF-IR, mTOR and Raf-1 with clinicopathological staging of BTC. The comparison of these proteins with clinicopathological staging is presented in Table III. To verify the association between the expression of these proteins and pT stage, the specimens were grouped as AJCC pathological stage I (early) vs. II-IV (advanced). For all the proteins, no significant association between early and advanced stage was observed in any of the BTC patients. As regards GBCa, the frequency of Raf-1-positive staining was significantly higher in advanced-stage compared to that in early-stage disease (Table III, 93 vs. 60%, P=0.03), whereas the expression of Raf-1 did not differ significantly by clinicopathological stage in IHBDCa and EHBDCa (data not shown).

Correlation of IGF-IR, mTOR and Raf-1 in BTC. To elucidate the associations between IGF-IR, mTOR and Raf-1 expressions, the positive frequency of any two of these proteins was evaluated. As shown in Table IV, the expression of IGF-IR and mTOR and the expression of Raf-1 and mTOR were correlated to some extent in all the subjects, although these correlations were not found to be statistically significant. Furthermore,

Table IV. Immunohistochemical co-expression rate and association between two proteins in biliary tract cancer.

Protein expression	mTOR		Raf-1		Raf-1	
	-	+	-	+	-	+
IGF-IR						
-	10	15	5	20		
+	11	42	7	46		
mTOR						
-					6	15
+					6	51
	P=0.07		P=0.32		P=0.05	

A two-sided χ^2 test was used for comparison between groups. mTOR, mammalian target of rapamycin; Raf-1, rapidly accelerated fibrosarcoma-1; IGF-IR, insulin-like growth factor type I receptor.

there were no correlations between these proteins when they were separately analyzed in GBCa, IHBDCa and EHBDCa (data not shown).

Survival analysis. The expression of IGF-IR, mTOR and Raf-1 was taken into consideration in the survival analysis. For IGF-IR, the 4-year survival rate of patients with positive expression was 32%, which was significantly lower compared to that of patients with negative expression (73%, P=0.024) (Fig. 2A). When each cancer site was analyzed individually, statistical significance was only demonstrated in IHBDCa. Cases with positive expression of IGF-IR tended to exhibit poorer prognosis compared to cases with negative expression (Fig. 2B-D). As regards mTOR and Raf-1, the 4-year survival rates were 52 and 49% in cases with positive expression vs. 50 and 64% in
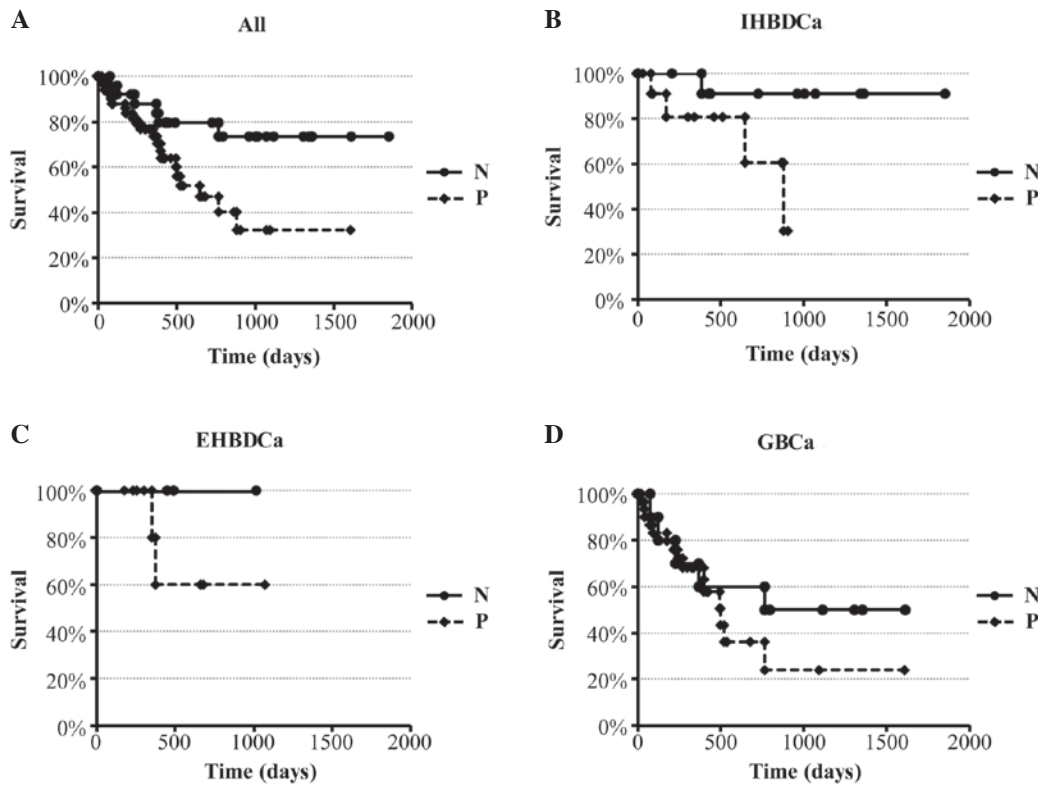
Figure 2. Kaplan-Meier curves according to insulin-like growth factor type I receptor (IGF-IR) expression in biliary tract cancer patients. (A) The 4-year survival rate of patients with positive IGF-IR expression was significantly lower compared to that of patients with negative expression. (B-D) Cases with positive expression of IGF-IR tended to exhibit poorer prognosis compared to cases with negative expression. N, negative IGF-IR expression; P, positive IGF-IR expression; IHBDCa, intrahepatic bile duct carcinoma; EHBDCa, extrahepatic bile duct carcinoma; GBCa, gallbladder carcinoma; (A) log-rank test P=0.024; (B) log-rank test P=0.049; (C) log-rank test P=0.246; and (D) log-rank test P=0.369.

cases with negative expression. However, there was no significant association between survival rate and positive expression of these two proteins when each cancer site was analyzed individually (data not shown).

Discussion

In this study, we demonstrated that i) IGF-IR, mTOR and Raf-1 are overexpressed in human BTC; ii) the expression of these proteins was not correlated to the histological type of the tumors; iii) the positive expression rate of Raf-1 was higher in advanced-stage compared to that in early-stage GBCa; and iv) the positive expression of IGF-IR was associated with poor prognosis in patients with resected BTC.

The insulin growth factor (IGF)-phosphoinositide 3 kinase (PI3K) pathway plays an important role in cell proliferation, apoptosis, migration and differentiation (13) (Fig. 3). This pathway is activated by interaction of IGF-IR, a transmembrane tyrosine kinase and its ligands, IGF-1 and IGF-2, leading to carcinogenesis and tumor progression by modulating cancer cell motility, adhesion (14) and angiogenesis (15). The expression rate of IGF-IR in our study was 68%, which is similar to that reported in other malignancies, including GBCa (16-18). If this high rate of IGF-IR expression in BTC indicates activation of the IGF-PI3K kinase pathway, there is the possibility of IGF-IR-targeted therapy, such as the use of a receptor-specific blocking monoclonal antibody and small-molecule tyrosine kinase inhibitors. Indeed, a previous clinical trial using a
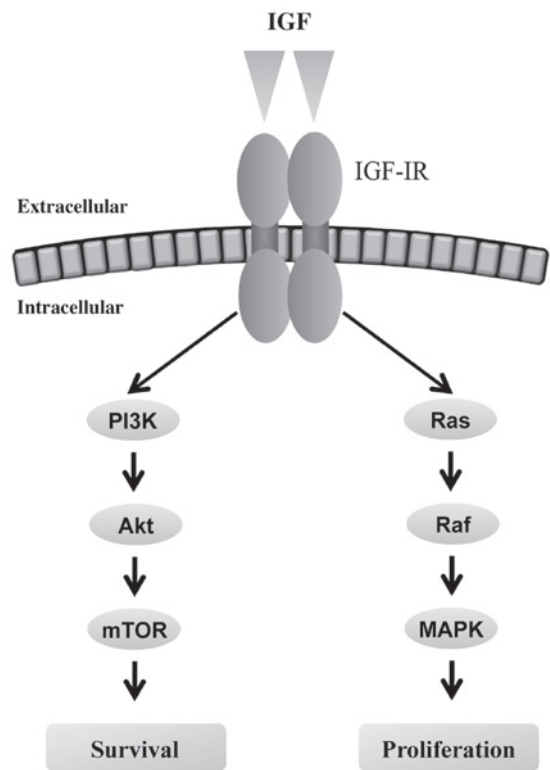

Figure 3. Major signaling pathways of insulin-like growth factor type I receptor (IGF-IR) in cancer cells. IGF, insulin-like growth factor; PI3K, phosphoinositide 3-kinase; Ras, rat sarcoma; Akt, protein kinase B; Raf, rapidly accelerated fibrosarcoma; mTOR, mammalian target of rapamycin; MAPK, mitogen-activated protein kinase.

monoclonal antibody against IGF-IR in solid tumors indicated its safety and efficacy (19). Our results demonstrated that there was no association between IGF-IR and pathological stage or histological grade, as shown in other types of cancer (13). By contrast, Ohashi *et al* (18) reported that IGF-IR expression was associated with pathological T stage. This may be explained by the distribution of each stage, such as the ratio of stage IV being 46% in our cases but only 7% in their cases. Of note, despite the fact that there was no difference in the rate of IGF-IR expression by pathological stage, patients with positive expression of IGF-IR exhibited worse survival. These results suggest that IGF-IR may be involved in cancer cell proliferation at an early stage and that prolonged expression of IGF-IR may contribute to rapid cancer growth and poor outcome.

To the best of our knowledge, this is the first study indicating that IGF-IR expression may predict unfavorable prognosis for the patients with BTC. Since surgery alone is rarely curative, the prognosis of human BTC is generally poor. The identification of a prognostic biomarker may help distinguish patients who may benefit from additional treatments in order to decrease the risk of recurrence. As mentioned above, an association between IGF-IR expression and poor clinical outcome has been demonstrated in other tumors. However, Kornprat *et al* (17) reported that low IGF-IR expression was an independent marker of poor prognosis. There is a limit to the argument regarding the association between molecular expression and patient prognosis, as prognosis depends on a number of factors.

mTOR is a serine/threonine kinase regulated by protein kinase B (Akt) and has been shown to integrate signaling from growth factors and nutrients and to regulate cell growth and cell cycle progression (20-22). Moreover, mTOR is overexpressed in a significant number of human tumors, either through upregulation of Akt or through alternative regulatory pathways (22). Activation of the Akt/mTOR signaling pathway may also result from enhanced HER2, activation since a significant percentage of human GB tumors have been shown to have positive expression of HER2 and/or EGFR (23-26). Leal *et al* (27,28) reported that phospho-mTOR was associated with poor prognosis and the Akt/mTOR substrate P70S6K is frequently phosphorylated in patients with GBCa. Our results revealed that ~70% of BTC samples expressed mTOR, which did not deviate from the IGF-IR expression rate. Indeed, we previously demonstrated the therapeutic effect of rapamycin for GBCa in BK5.erbB2 transgenic mice, which have an extremely high incidence of GBCa (29).

Raf-1 is a critical mediator of mitogenic signals emanating from a variety of receptor tyrosine kinases, including EGFR and HER2 (30). Raf-1 activation of the mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase pathway inhibits apoptosis in cancer cells and MEK inhibitors may abrogate the antiapoptotic function of Raf-1 (31,32). There are only episodic reports of Raf-1-positive expression, particularly in ovarian and head and neck cancer (33,34). Although the roles of Raf-1 in BTC carcinogenesis and progression are not fully understood, our results, demonstrating a high rate of Raf-1 expression, may indicate activation of the IGFR-IR/Ras/Raf pathway.

There were several limitations to this study. First, our results were from IHC of surgical specimens. The protein or mRNA elevation was not investigated by western blotting or polymerase chain reaction methods. Second, the phosphorylation of IGF-IR, mTOR and Raf-1 was not analyzed. Finally, the methodology of IHC and the interpretation of the results differ among institutions. To solve these problems, further studies with sufficient numbers of samples are required.

In summary, IGF-IR, mTOR and Raf-1 were found to be highly expressed in BTC and targeted therapy against IGF-IR may be effective in BTC patients. In addition, the high expression of IGF-IR exhibited a significant association with poor prognosis, indicating that IGF-IR may be a useful biomarker for predicting prognosis.

Acknowledgements

References

1. Jarnagin WR, Ruo L, Little SA, *et al*: Patterns of initial disease recurrence after resection of gallbladder carcinoma and hilar cholangiocarcinoma: implications for adjuvant therapeutic strategies. Cancer 98: 1689-1700, 2003.
2. Shaib YH, Davila JA, McGlynn K and El-Serag HB: Rising incidence of intrahepatic cholangiocarcinoma in the United States: a true increase? J Hepatol 40: 472-477, 2004.
3. Jemal A, Murray T, Ward E, *et al*: Cancer statistics, 2005. CA Cancer J Clin 55: 10-30, 2005.
4. Randi G, Franceschi S and La Vecchia C: Gallbladder cancer worldwide: geographical distribution and risk factors. Int J Cancer 118: 1591-1602, 2006.
5. Roa I, Araya JC, Villaseca M, Roa J, de Aretxabala X and Ibacache G: Gallbladder cancer in a high risk area: morphological features and spread patterns. Hepatogastroenterology 46: 1540-1546, 1999.
6. Medina E and Kaempffer AM: Cancer mortality in Chile: epidemiological considerations. Rev Med Chil 129: 1195-1202, 2001 (In Spanish).
7. Kimura K, Ohto M, Saisho H, *et al*: Association of gallbladder carcinoma and anomalous pancreaticobiliary ductal union. Gastroenterology 89: 1258-1265, 1985.
8. Kawamoto T, Krishnamurthy S, Tarco E, *et al*: HER receptor family: novel candidate for targeted therapy for gallbladder and extrahepatic bile duct cancer. Gastrointest Cancer Res 1: 221-227, 2007.
9. Watanabe H, Date K, Itoi T, *et al*: Histological and genetic changes in malignant transformation of gallbladder adenoma. Ann Oncol 10 (Suppl 4): 136-139, 1999.
10. Furstenberger G and Senn HJ: Insulin-like growth factors and cancer. Lancet Oncol 3: 298-302, 2002.
11. Papa V, Gliozzo B, Clark GM, *et al*: Insulin-like growth factor-I receptors are overexpressed and predict a low risk in human breast cancer. Cancer Res 53: 3736-3740, 1993.
12. Matsubara J, Yamada Y, Hirashima Y, *et al*: Impact of insulin-like growth factor type 1 receptor, epidermal growth factor receptor, and HER2 expressions on outcomes of patients with gastric cancer. Clin Cancer Res 14: 3022-3029, 2008.
13. LeRoith D and Roberts CT Jr: The insulin-like growth factor system and cancer. Cancer Lett 195: 127-137, 2003.
14. Mauro L and Surmacz E: IGF-I receptor, cell-cell adhesion, tumor development and progression. J Mol Histol 35: 247-253, 2004.
15. Reinmuth N, Fan F, Liu W, *et al*: Impact of insulin-like growth factor receptor-I function on angiogenesis, growth, and metastasis of colon cancer. Lab Invest 82: 1377-1389, 2002.
16. Imsumran A, Adachi Y, Yamamoto H, *et al*: Insulin-like growth factor-I receptor as a marker for prognosis and a therapeutic target in human esophageal squamous cell carcinoma. Carcinogenesis 28: 947-956, 2007.

17. Kornprat P, Rehak P, Ruschoff J and Langner C: Expression of IGF-I, IGF-II, and IGF-IR in gallbladder carcinoma. A systematic analysis including primary and corresponding metastatic tumors. J Clin Pathol 59: 202-206, 2006.
18. Ohashi H, Adachi Y, Yamamoto H, et al: Insulin-like growth factor receptor expression is associated with aggressive phenotypes and has therapeutic activity in biliary tract cancers. Cancer Sci 103: 252-261, 2012.
19. Karp DD, Paz-Ares LG, Novello S, et al: Phase II study of the anti-insulin-like growth factor type 1 receptor antibody CP-751,871 in combination with paclitaxel and carboplatin in previously untreated, locally advanced, or metastatic non-small-cell lung cancer. J Clin Oncol 27: 2516-2522, 2009.
20. Asnaghi L, Bruno P, Priulla M and Nicolin A: mTOR: a protein kinase switching between life and death. Pharmacol Res 50: 545-549, 2004.
21. Richardson CJ, Schalm SS and Blenis J: PI3-kinase and TOR: PIKTORing cell growth. Semin Cell Dev Biol 15: 147-159, 2004.
22. Wullschleger S, Loewith R and Hall MN: TOR signaling in growth and metabolism. Cell 124: 471-484, 2006.
23. Ito Y, Takeda T, Sasaki Y, et al: Expression and clinical significance of the erbB family in intrahepatic cholangiocellular carcinoma. Pathol Res Pract 197: 95-100, 2001.
24. Yukawa M, Fujimori T, Hirayama D, et al: Expression of oncogene products and growth factors in early gallbladder cancer, advanced gallbladder cancer, and chronic cholecystitis. Hum Pathol 24: 37-40, 1993.
25. Suzuki H, Isaji S, Pairojkul C and Uttaravichien T: Comparative clinicopathological study of resected intrahepatic cholangiocarcinoma in northeast Thailand and Japan. J Hepatobiliary Pancreat Surg 7: 206-211, 2000.
26. Ukita Y, Kato M and Terada T: Gene amplification and mRNA and protein overexpression of c-erbB-2 (HER-2/neu) in human intrahepatic cholangiocarcinoma as detected by fluorescence in situ hybridization, in situ hybridization, and immunohisto-chemistry. J Hepatol 36: 780-785, 2002.
27. Leal P, Garcia P, Sandoval A, et al: Immunohistochemical expression of phospho-mTOR is associated with poor prognosis in patients with gallbladder adenocarcinoma. Arch Pathol Lab Med 137: 552-557, 2013.
28. Leal P, Garcia P, Sandoval A, et al: AKT/mTOR substrate P70S6K is frequently phosphorylated in gallbladder cancer tissue and cell lines. Onco Targets Ther 6: 1373-1384, 2013.
29. Wu Q, Kiguchi K, Kawamoto T, et al: Therapeutic effect of rapamycin on gallbladder cancer in a transgenic mouse model. Cancer Res 67: 3794-3800, 2007.
30. Kolch W: Meaningful relationships: the regulation of the Ras/Raf/MEK/ERK pathway by protein interactions. Biochem J 351: 289-305, 2000.
31. Cleveland JL, Troppmair J, Packham G, et al: v-raf suppresses apoptosis and promotes growth of interleukin-3-dependent myeloid cells. Oncogene 9: 2217-2226, 1994.
32. Erhardt P, Schremser EJ and Cooper GM: B-Raf inhibits programmed cell death downstream of cytochrome c release from mitochondria by activating the MEK/Erk pathway. Mol Cell Biol 19: 5308-5315, 1999.
33. McPhillips F, Mullen P, Monia BP, et al: Association of c-Raf expression with survival and its targeting with antisense oligo-nucleotides in ovarian cancer. Br J Cancer 85: 1753-1758, 2001.
34. Riva C, Lavieille JP, Reyt E, Brambilla E, Lunardi J and Brambilla C: Differential c-myc, c-jun, c-raf and p53 expression in squamous cell carcinoma of the head and neck: implication in drug and radioresistance. Eur J Cancer B Oral Oncol 31B: 384-391, 1995.