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MUC4 interacts with ErbB2 in human gallbladder carcinoma: Potential pathobiological implications

Naoki Miyahara[a,b], Junichi Shoda[a,], Kazunori Ishige[a], Toru Kawamoto[c], Tetsuya Ueda[d], Ryuuichi Taki[e], Nobuhiro Ohkohchi[f], Ichinosuke Hyodo[a], Melanie B. Thomas[c], Savitri Krishnamurthy[g], Kermit L. Carraway[h], Tatsuro Irimura[b]*

[a]*Department of Gastroenterology, Institute of Clinical Medicine, University of Tsukuba Graduate School of Comprehensive Human Sciences, 1-1-1 Tennodai, Tsukuba-shi, Ibaraki 305-8575, Japan*
[b]*Laboratory of Cancer Biology and Molecular Immunology, Graduate School of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo, Japan*
[c]*Department of Medical Oncology, University of Texas, M.D. Anderson Cancer Center, Houston, TX, United States*
[d]*Bioanalysis Department, Drug Development Service Division, Mitsubishi Chemical Medience Corporation, Itabashi-ku, Tokyo, Japan*
[e]*Japan Cytology and Pathology Laboratory Inc., Subsidiary of Mitsubishi Chemical Medience Corporation, Itabashi-ku, Tokyo, Japan*
[f]*Department of Surgery, Institute of Clinical Medicine, University of Tsukuba Graduate School of Comprehensive Human Sciences, Tsukuba-shi, Ibaraki, Japan*
[g]*Department of Cytology, University of Texas, M.D. Anderson Cancer Center, Houston, TX, United States*
[h]*Department of Cell Biology and Anatomy, University of Miami School of Medicine, Miami, FL, United States*

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ABSTRACT

Muc4 interacts with erbB2 and potentiates tumourigenesis and/or tumour growth. The expression of MUC4, the interaction of MUC4 with erbB2 and the status of erbB2 signalling in human gallbladder carcinomas were determined in order to gain a better understanding of the pathobiology. The expression levels of MUC4 protein and mRNA were increased in specimens of gallbladder carcinoma. Immunoprecipitation experiments showed an interaction between MUC4 and erbB2. This interaction was associated with the hyperphosphorylation of erbB2, MAPK and Akt and with the overexpression of cyclooxygenase-2. MUC4 was detected on the apical surface of cancerous epithelia and partially co-localised there with erbB2. Transfection experiments showed that MUC4 amplifies cell proliferation in the presence of heregulin through potentiating phosphorylation of erbB2 and its downstream signalling pathways. These findings suggest that MUC4 is up-regulated and interacts with erbB2 in human gallbladder carcinoma, and thereby support the potential implication of MUC4 in erbB2 activation.

1. Introduction

Nearly two-thirds of biliary tract carcinomas arise in the gallbladder, making gallbladder carcinoma the most common biliary tract carcinoma. Gallbladder carcinoma has always been associated with a dismal overall prognosis.[1,2] This is essentially attributed to slow and asymptomatic growth of gallbladder carcinoma infiltrating the surrounding structures, and the disease is therefore usually detected at an advanced stage with a high frequency of distant organ metastasis. Details of

* *Corresponding author:* Tel./fax: +81 29 853 3324.
E-mail address: shodaj@md.tsukuba.ac.jp (J. Shoda).
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tumourigenesis as well as growth and progression of the disease are complex and not completely understood. Certain predisposing factors such as chronic cholecystitis, obesity and the presence of an anomalous pancreaticobiliary junction have been linked to the disease.[3] Genetic alterations in *p53* or *K-ras* may contribute to the development of certain types of gallbladder carcinoma.[4,5] Overexpression of erbB2 and/or EGFR, oncogenes, has been reported in a significant percentage of gallbladder carcinomas.[6–8]

BK5.erbB2 transgenic mice that overexpress wild-type rat *erbB2* under the control of the *bovine keratin* 5 *(BK5)* promoter have been generated and found to develop adenocarcinoma of the gallbladder at a high incidence.[9] In this model of gallbladder carcinoma, the results of *in vitro*[10] and *in vivo* experiments[11,12] have suggested that alterations in erbB2 signalling have been implicated in neoplastic transformation. We have recently shown that Muc4, a membrane mucin, is up-regulated and interacts with erbB2 in BK5.erbB2 mice.[13] The observation suggests that Muc4 may play an important role during gallbladder carcinogenesis through potentiating erbB2 signalling.

Several studies have recently shown the involvement of membrane mucins such as Muc1 and Muc4 in cell signalling.[14–16] Structures of Muc4 show the transmembrane subunit ASGP2 and the mucin subunit ASGP1.[16] ASGP2 contains two epidermal growth factor (EGF) domains with conserved amino acid residues of active EGF-like growth factors, one of which reportedly acts as a ligand for erbB2.[16] Thus, Muc4 acts as a novel transmembrane ligand for the tyrosine kinase erbB2, triggering specific phosphorylation of erbB2.[17] The expression of MUC4 has been reported in human biliary tract carcinomas.[18,19] In terms of pathobiology for biliary carcinogenesis, these findings can be combined with *erbB2* amplification and/or overexpression in biliary tract carcinomas.[20]

In this study, we investigated the validity of the hypothesis that the up-regulation of MUC4 and its interaction with erbB2 in human gallbladder carcinoma are involved in the process of the carcinogenesis through potentiating erbB2 signalling.

2. Materials and methods

2.1. Patients

Specimens from 51 patients (18 males and 33 females) with gallbladder carcinoma (5 with pT1, 17 with pT2, 5 with pT3 and 24 with pT4 carcinomas) were included in this study. The mean age of the patients was 62 years (range, 44–76 years). The patients were diagnosed as having gallbladder carcinoma and underwent operations in the Hospital of the University of Tsukuba. Gallbladder carcinoma was diagnosed on the basis of histological findings and was classified according to the tumour node metastasis (TNM) classification of the American Joint Committee on Cancer (AJCC).[21] In addition, gallbladder specimens obtained at surgery from 7 subjects with gallstones who had undergone cholecystectomy were used as intact gallbladder specimens. The study protocol was performed according to the principles of the Declaration of Helsinki, and informed consent was obtained from all patients.

2.2. Real-time quantitative polymerase chain reaction

Steady-state mRNA levels were determined by real-time quantitative PCR. Primers and probes for human *MUC4 (ASGP2)* and *erbB2* are summarised in Table 1. In each experiment, PCR was done in triplicate. The PCR data were expressed relative to the amount of *rRNA* present in each specimen and then averaged.

2.3. Immunoblot analysis

Immunoblot analysis was performed using the lysates prepared from frozen tissue specimens of 12 gallbladder carcinoma cases. Capan-1, a pancreatic carcinoma cell line, was used as a positive control. Tissue lysates and immunoprecipitates, which were prepared as described previously[11], were electrophoresed through 7–10% SDS/polyacrylamide gels and transferred to polyvinylidene difluoride membranes. After blocking with 1% non-fat powdered milk in PBST (0.05% Tween in PBS), the protein levels of MUC4 (ASGP2), erbB2, p-erbB2, MAPK, p-MAPK, Akt and p-Akt were detected by incubating the membrane with the corresponding anti-MUC4 (ASGP2) antibody (Ab) (Zymed Laboratories Inc., South San Francisco, CA), anti-erbB Abs (Cell Signaling Technology, Beverly, MA), anti-MAPK Abs (Cell Signaling Technology) or anti-Akt Abs (Cell Signaling Technology). Protein bands were visualised as described previously.[12]

2.4. Immunohistochemistry

For the immunostaining of MUC4, the 2-μm-thick tissue sections were stained by the indirect immunoperoxidase method using anti-MUC4 (ASGP2) Ab. A negative control was made using bovine serum albumin instead of anti-MUC4 Ab.

For the immunostaining of erbB2, HercepTest (Dako A/S, Glastrup, Denmark) was performed following the manufacturer's instructions. The breast cancer cells supplied with this kit were used as positive and negative control specimens.

Immunohistochemical evaluation was performed independently by two investigators with respect to the histopathological characteristics and specific immunoreactivity. The status of MUC4 was evaluated by the percentage of positively

Table 1 – Primer and double-dye-probe sets used for real-time quantitative PCR

Name	Sequence
MUC4 forward primer	5′-CAG CCCAAGCTATAGTGTGAT-3′
MUC4 reverse primer	5′-TGATGGTGCCGTTGTAATTTG-3′
MUC4 probe	FAM5′-CCACATCCCCATCTTCTTCACCTATGCTG-3′ TAM
erbB2 forward primer	5′-CTGAACTGGTGTATGCAGATTGC-3′
erbB2 reverse primer	5′-GTAATTTTGACATGGTTGGGACTCT-3′
erbB2 probe	FAM5′-TGGAGGATGTGCGGCTCGTACACAG-3′TAM

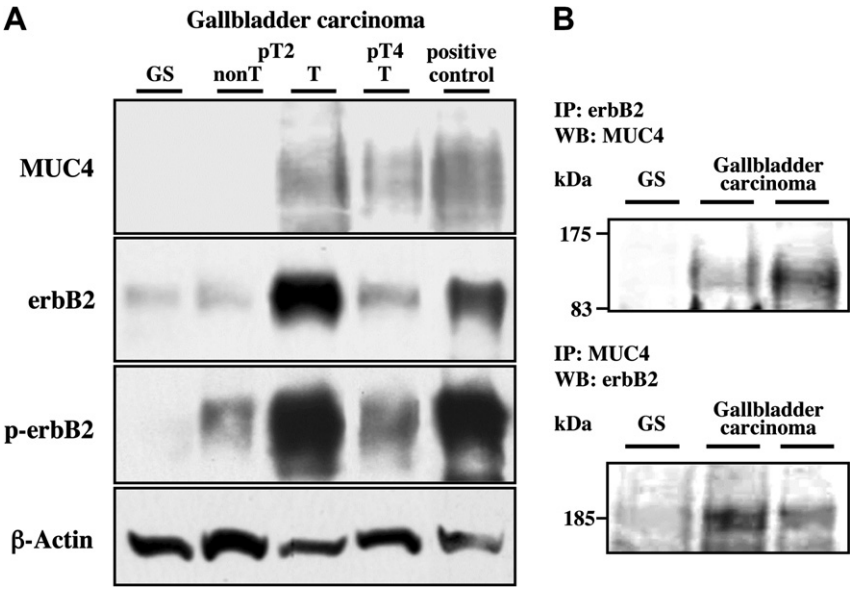

Fig. 1 – Immunoblot analysis of MUC4 and erbB2 (A) and interaction of MUC4 and erbB2 (B) in gallbladder carcinoma tissues and gallbladders with gallstones (GS). Protein was normalised to β-actin. Capan-1, a pancreatic carcinoma cell line, was used as a positive control.

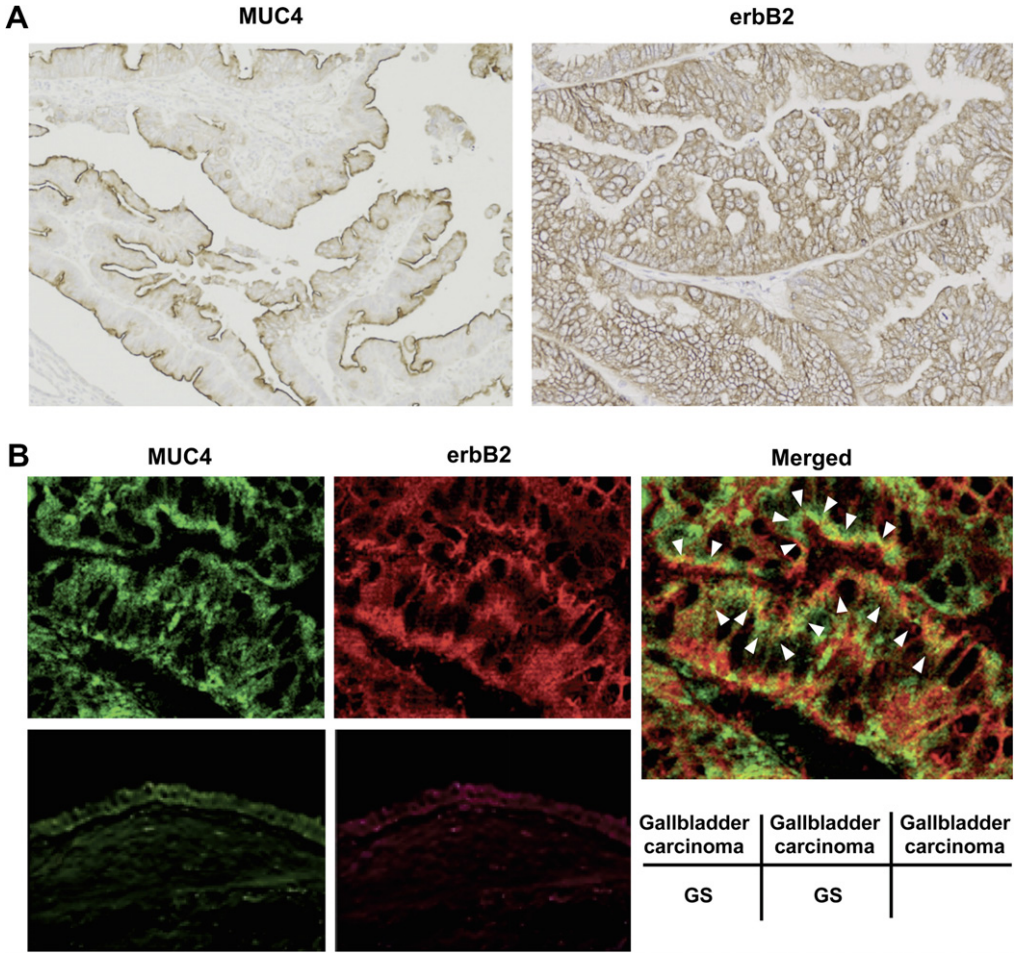

Fig. 2 – Indirect immunoperoxidase staining (A, original magnification ×33) and immunofluorescent staining (B, ×198) of MUC4 and erbB2 in the tissue sections of gallbladder carcinoma and gallbladders with gallstones (GS). MUC4 (green) was partially co-localised (yellow) with erbB2 (red) in the cancerous epithelia.

stained carcinoma cells. Membranous expression of MUC4 was evaluated. According to our previous study[22], the staining of MUC4 was judged as positive when more than 10% of the total number of cancerous epithelia in each section showed MUC4 expression in the membrane. For the evaluation of erbB2 status, only the membranous staining intensity and pattern were evaluated according to the HercepTest scoring system. Cases in which there was no staining or membrane staining in less than 10% of cancerous or non-cancerous cells were scored as 0. A faint/barely perceptible membrane staining in more than or equal to 10% of cancerous or non-cancerous cells where only part of the membrane stained was scored as 1+. A weak to moderate complete membrane staining in more than 10% of the cancerous cells was scored as 2+. A strong complete membrane staining in more than 10% of the cancerous cells was scored as 3+. ErbB2 staining was interpreted as negative (0 and 1+) and positive (2+ and 3+) for each protein overexpression.

Double immunofluorescence staining was performed to determine the status of co-localisation of MUC4 and erbB2 using 4-μm-thick tissue sections. The slides were incubated with anti-MUC4 Ab or with anti-erbB2 Ab. The slides were subsequently incubated with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG + IgM Ab or Cy3-conjugated donkey rabbit IgG Ab diluted. Images were acquired from two channels at wavelengths of 488 nm and 543 nm using a confocal scanning laser microscope (TCS-SP2, Leica Lasertechnik GmbH, Wetzlar, Germany).

2.5. *Fluorescence in situ hybridisation (FISH)*

The number of erbB2 gene copies was determined by the *FISH* method using a Vysis Path Vysion HER-2/DNA probe kit (Vysis, Downers Grove, IL). Briefly, the slides were baked overnight at 56 °C. The erbB2/DNA and chromosome 17 pre-mixed probe was applied to the target area on each slide. Denaturation of the sample DNA and probe DNA was performed at 73 °C for 5 min and the slides were incubated overnight at 37 °C. Then, the slides were counterstained with 4,6-diaminidino-2-phenyl-indole (DAPI). Signal enumeration was conducted at 1000× magnification with the appropriate filter. ErbB2 gene amplification was defined as a LSI HER-2/chromosome 17 (CEP 17) ratio of 2.0 or greater. Several fields of at least 20 cells were counted and the results were averaged.

2.6. *Cell lines and culture conditions*

A375 human melanoma cell lines expressing Muc4/sialomucin complex under tetracycline regulation,[23] which were developed by Dr. K. L. Carraway, a co-author of this study,

Table 2 – Summary of Data on the Expression of MUC4 and erbB2 and Clinicopathological Features of 51 Cases of Gallbladder Carcinoma

Gallbladders	MUC4 (ASGP2)		erbB2		MUC4/ erbB2
	+	−	+	−	+ / +
Chronic cholecystitis (n = 7)	0 (0)	7 (100)	1 (14)[c]	6 (86)	0 (0)
Carcinomas (n = 51)	34 (67)	17 (33)	16 (31)	35 (69)	12 (24)[e]
Histology					
Well (*n* = 17)[a]	13 (76)	4 (24)	6 (35)	11 (65)	5 (29)
Mod (*n* = 26)	19 (73) [d]	7 (27) [d]	8 (31)	18 (69)	6 (23)
Poor (*n* = 8)	2 (25)	6 (75)	2 (25)	6 (75)	1 (13)
Depth of invasion					
pT1 (*n* = 5)[b]	4 (75)	1 (25)	2 (40)	3 (60)	2 (40)
pT2 (*n* = 17)	14 (82)	3 (18)	6 (35)	11 (65)	5 (29)
pT3 (*n* = 5)	3 (60)	2 (40)	1 (20)	4 (80)	1 (20)
pT4 (*n* = 24)	10 (42)	14 (58)	7 (29)	17 (71)	4 (17)
Distant organ metastasis					
+ (*n* = 18)	12 (67)	6 (33)	6 (33)	12 (67)	4 (22)
− (*n* = 33)	22 (67)	11 (33)	10 (30)	23 (70)	8 (24)
Lymphatic permeation					
+ (*n* = 32)	21 (66)	11 (34)	9 (28)	23 (72)	7 (22)
− (*n* = 19)	13 (68)	6 (32)	6 (32)	13 (68)	5 (26)
Venous permeation					
+ (*n* = 22)	12 (55)	10 (45)	7 (32)	15 (68)	5 (23)
− (*n* = 29)	22 (76)	7 (24)	8 (28)	21 (72)	7 (24)
Lymph node metastasis					
+ (*n* = 21)	14 (67)	7 (33)	6 (29)	15 (71)	5 (21)
− (*n* = 30)	20 (67)	10 (33)	9 (30)	21 (70)	7 (23)

a Well, well-differentiated; mod, moderately differentiated; poor, poorly differentiated.
b pT, pathological tumor stage.
c Values in parentheses represent percentages.
d $P < 0.05$, significantly different between the two groups.
e $P < 0.01$, significantly different from other groups (MUC4−/erbB2+, MUC4+/erbB2− and MUC4−/erbB2−).

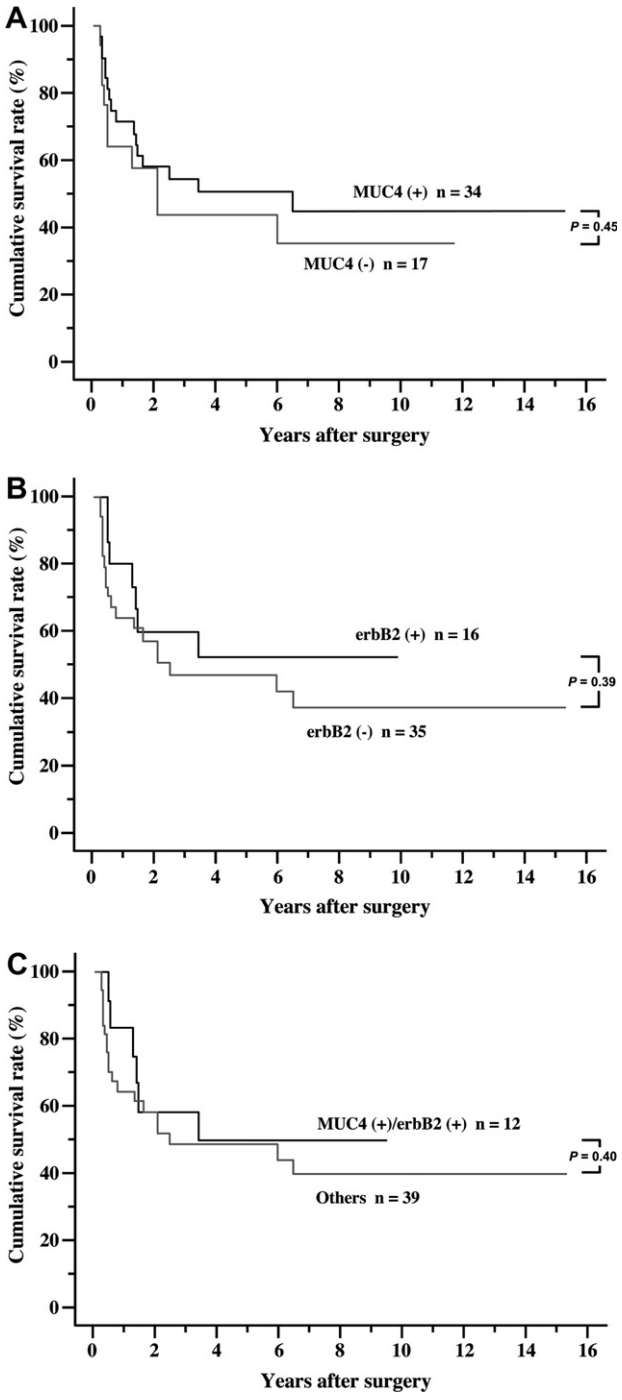

Fig. 3 – Relationship between the survival outcome and the expression status of MUC4 and erbB2 (MUC4-positive (+) versus negative (–), erbB2+ versus erbB2–, both MUC4+/erbB2+ versus MUC4–/erbB2+, MUC4+/erbB2– plus MUC4–/erbB2-) in 51 surgical specimens of gallbladder carcinoma by Kaplan–Meier's survival curves.

were used. Under a serum-starved condition, A375 human melanoma cell transfectants were treated or not treated with recombinant heregulin EGF-like domain (10 nmol/L) for 5 min. The protein levels of Muc4 (ASGP2), erbB2, p-erbB2, MAPK, p-MAPK, Akt and p-Akt were detected by incubating the membranes with the Abs. In addition, the cells were treated with heregulin (0.1–10 nmol/L) and then the incorporation of [^{3}H]thymidine into DNA was determined as described previously.[24]

2.7. Statistics

Values are given as means ± SEM (standard error of the mean). Means of the two groups were compared with the Mann–Whitney rank sum *U*-test (two-tailed test), and multiple comparisons were performed by ANOVA. A two-sided X^2 test was used for comparison of clinicopathological data between groups. Correlation was tested by calculating Spearman's rank-order correlation coefficient, *r* (two-tailed test). The survival of patients was recorded every month, and patient survival was analysed by the method of Kaplan–Meier. Differences in the survival of patients in subgroups were analysed by the log-rank test. A *P* value of <0.05 was defined as statistically significant.

3. Results

3.1. Expression status of MUC4 and ErbB2 in human gallbladder carcinoma

MUC4 (ASGP2) was detected in the lysates of gallbladder carcinoma tissues (Fig. 1A). Eight (67%) of 12 cases of gallbladder carcinoma were found to yield immunoreactive bands of MUC4. In the 7 specimens of gallbladders associated with gallstones, no bands of MUC4 were detected. ErbB2 was also detected in the lysates of gallbladder carcinoma tissues (Fig. 1A). Four (33%) of 12 cases of gallbladder carcinoma were found to yield immunoreactive bands of both erbB2 and phosphorylated erbB2. However, in the 7 specimens of gallbladders associated with gallstones, only trace or no immunoreactive bands of erbB2 were found and no bands of phosphorylated erbB2 were detected.

The tissue lysates of the four cases of gallbladder carcinoma that expressed both MUC4 and erbB2 on immunoblots were further analysed by immunoprecipitation and then immunoblotted to determine a direct interaction of MUC4 and erbB2. As shown in Fig. 1B, the experiments revealed a direct interaction between MUC4 and erbB2 in human gallbladder carcinoma tissues as was found in human breast carcinoma.[25]

3.2. Immunohistochemical localisations of MUC4 and ErbB2 in human gallbladder carcinoma

MUC4 was strongly expressed in the apical membranous components of the carcinoma epithelia and/or in the cytoplasm (for parts of the cases studied) (Fig. 2A) but not in the membranous components of the gallbladders associated with gallstones. On the other hand, erbB2 was expressed in the membranous components of the carcinoma epithelia (Fig. 2A) but partly and only weakly in the membranous components of the gallbladders associated with gallstones (data not shown). In all of the 12 cases of gallbladder carcinoma that yielded both MUC4 and erbB2 on immunoblot analysis, double immunofluorescence stainings revealed that MUC4 and erbB2 were partly co-localised in the apical surfaces of the cancerous epithelia (Fig. 2B).

3.3. Relationship between pathological features and MUC4 or ErbB2 expression in human gallbladder carcinoma

MUC4 (ASGP2) was not expressed in the non-cancerous epithelia of gallbladders associated with gallstones, whereas positive immunostaining of erbB2 was seen in the epithelia in 1 (14%) of the 7 cases (Table 2). In the cancerous epithelia, positive stainings of MUC4 and erbB2 were seen in 34 (67%) and 16 (31%) of the 51 gallbladder carcinomas, respectively (Table 2). Correlating MUC4 with erbB2 expression, 12 (24%) of the 51 gallbladder carcinomas (5 for well, 6 for moderately and 1 for poorly differentiated adenocarcinomas) were found to have positive stainings of both MUC4 and erbB2. While the expression rate of MUC4 was significantly higher in the cases of well or moderately differentiated carcinomas than that in cases of the poorly differentiated carcinomas, no other significant differences in the expression rates of MUC4 and erbB2 were found with respect to histological grade, distant organ metastasis and pathological features (Table 2). A comparison of the survival outcome of the 51 patients with respect to the expression status of MUC4 and erbB2 was made, and the results revealed that the survival outcome of the patients was not significantly different between MUC4-positive and -negative groups ($P = 0.45$), between erbB2-positive and -negative groups ($P = 0.39$) and between MUC4/erbB2-both positive and the other groups ($P = 0.40$) as shown in Fig. 3.

3.4. Gene expression levels of MUC4 and ErbB2 in gallbladder carcinoma

The mRNA levels of MUC4 were significantly higher in the specimens of gallbladder carcinomas (30.5 ± 8.4%; $P < 0.01$) than that in the adjacent noncancerous tissues (6.4 ± 2.6%) and in the specimens of gallbladders associated with gallstones (1.0 ± 0.2%) (Fig. 4A). The mRNA levels tended to be higher in the specimens of pT1–pT2 carcinomas (46.8 ± 13.3%) than that in the specimens of pT3–pT4 carcinomas (21.2 ± 10.4%). With respect to the histological grade, the mRNA levels were significantly higher in the specimens of well differentiated adenocarcinoma (59.0 ± 15.6%; $P < 0.05$) than that in the specimens of poorly differentiated carcinoma (6.5 ± 2.7%). The mRNA levels in the specimens of moderately differentiated carcinoma (27.0 ± 12.0%) were intermediate between the levels in the specimens of well and poorly differentiated carcinoma.

On the other hand, the mRNA levels of erbB2 were significantly higher in the tissue specimens of gallbladder carcinoma (6.5 ± 1.6% of rRNA, mean ± SEM; $P < 0.01$) than that in the adjacent noncancerous tissues (1.2 ± 0.3%) and in the specimens of gallbladders associated with gallstones (1.0 ± 0.2%) (Fig. 4A). The mRNA levels were not significantly different between the specimens of pT1–pT2 (9.8 ± 3.4%) and pT3–pT4 (4.6 ± 1.5%) carcinomas or with respect to the histological grade (well, 8.4 ± 3.1%; moderate, 7.1 ± 2.4%; poor,

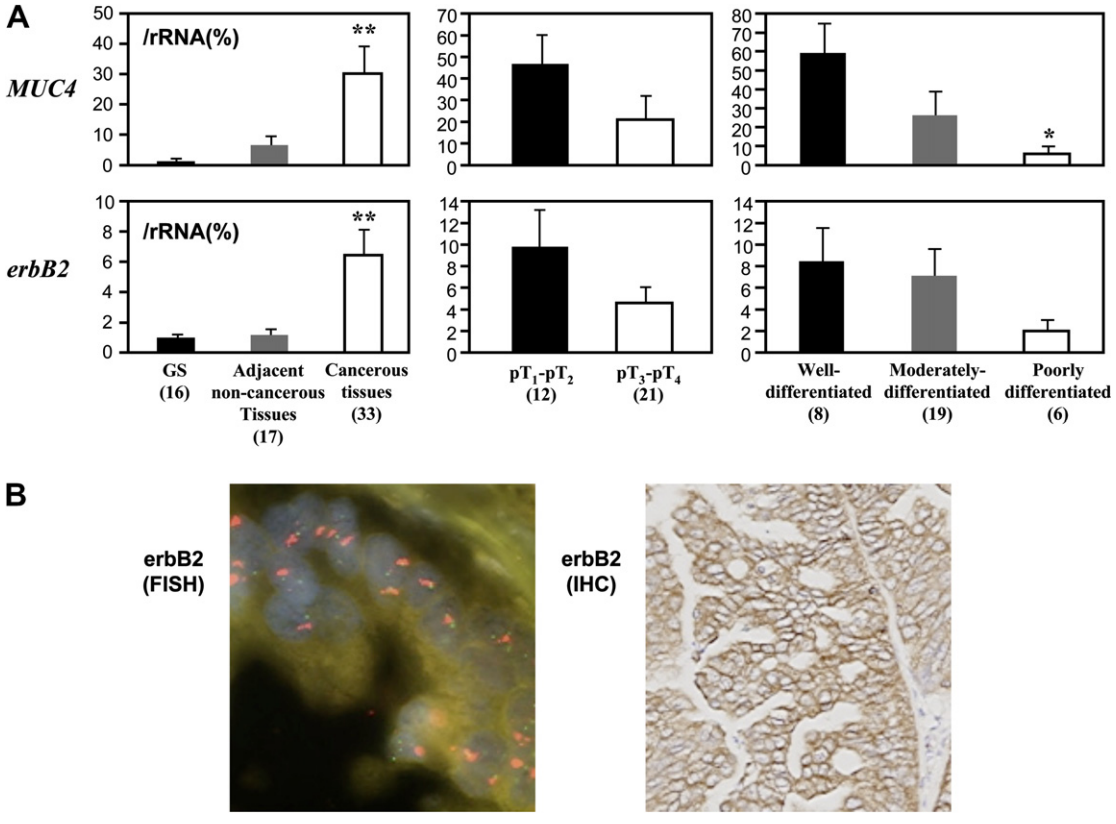

Fig. 4 – mRNA levels of *MUC4* and *erbB2* (A) and fluorescence in situ hybridisation (*FISH*, original magnification ×330) and immunohistochemistry (IHC, ×66) of erbB2 (B) in gallbladder carcinoma tissues and gallbladders with gallstones (GS). **P < 0.01, different from the GS- group; *P < 0.05, different from the well differentiated carcinoma group.

1.9 ± 0.5%). The gene amplification of erbB2 by *FISH* (Fig. 4B) was recognised in 4 of the 16 gallbladder carcinoma tissues (25%) that showed erbB2 overexpression by immunohistochemistry.

Based on the above data, because of a positive correlation between MUC4 and erbB2 mRNA levels in the specimens of gallbladder carcinomas ($n = 33$, $r = 0.72$, $P < 0.01$), more emphasis should be placed on the apparent closely paralleled patterns of MUC4 mRNA relative to that determined for erbB2 in human gallbladder carcinoma.

3.5. *Expression status of ErbB2-downstream molecules MAPK, Akt and COX-2 in human gallbladder carcinoma*

Based on the observation that in gallbladders of BK5.erbB2 mice hyperphosphorylation of erbB2, which interacts with Muc4, is associated with hyperphosphorylation of MAPK and Akt as well as Cox-2 overexpression; the activation of these erbB2-related molecules in human gallbladder carcinoma was investigated in the 4 cases that yielded both MUC4 and erbB2 bands on immunoblots.

As shown in Fig. 5, in terms of erbB2-downstream molecules, hyperphosphorylation levels of MAPK and Akt were significantly elevated in the specimens of gallbladder carcinoma. Moreover, in parallel to the activation of these molecules, Cox-2 protein levels were significantly elevated in the specimens of gallbladder carcinoma. Similar to the status in the gallbladder of BK5.erbB2 mice, hyperphosphorylation of MAPK and Akt and overexpression of Cox-2 were observed in all of the four cases studied.

3.6. *Biological effects of Muc4 on ErbB2 signalling and cell proliferation*

To investigate the biological role of Muc4 in gallbladder carcinogenesis, biological effects of Muc4 on the activation and modulation of erbB2 phosphorylation and signalling as well as cell proliferation were determined using A375 human melanoma cell transfectants overexpressing Muc4.[23]

The growth response of the A375 transfectants to heregulin, an EGF-like growth factor, which was determined by [^{3}H]thymidine uptake, was observed to be potentiated in a dose-dependent manner (Fig. 6A). Moreover, in the heregulin-stimulated proliferative response of these cells, the presence of Muc4 potentiated the heregulin effects on the phosphorylation of erbB2, MAPK and Akt (Fig. 6B).

4. Discussion

In human gallbladder carcinoma, gene expression levels of MUC4 apparently paralleled those of erbB2 (Fig. 4). Protein levels of MUC4 were greatly increased in 8 (67%) of the 12 tissue specimens of human gallbladder carcinoma studied (Fig. 1). Little or no Muc4 protein was detected in the adjacent noncancerous tissues and in the specimens of gallbladders associated with gallstones. Four (33%) of the 12 tissue specimens showed increased protein levels of erbB2 and its hyperphosphorylated form (Fig. 1), and 4 (25%) of the 16 specimens with erbB2 overexpression revealed by immunohistochemistry

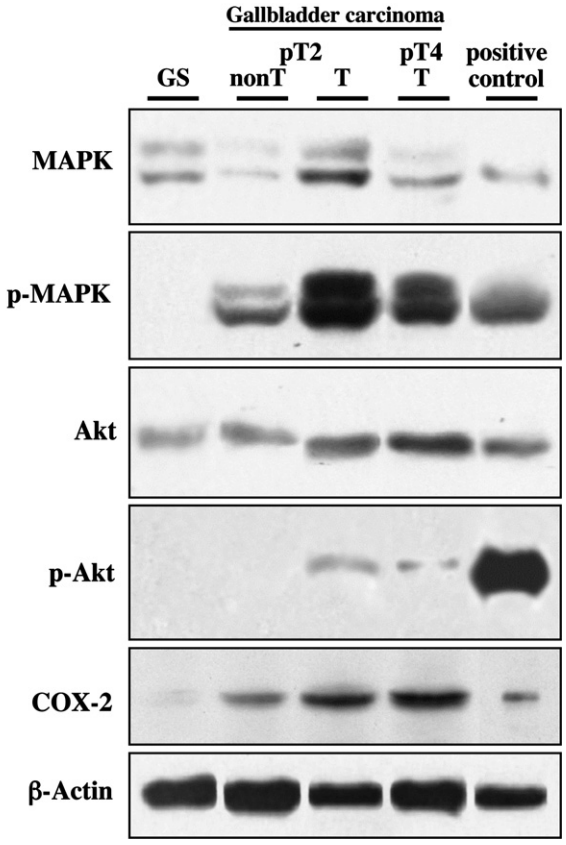

Fig. 5 – Immunoblot analysis of MAPK, Akt and Cox-2 in gallbladder carcinoma tissues and gallbladders with gallstones (GS). Protein was normalised to β-actin. Capan-1, a pancreatic carcinoma cell line, was used as a positive control.

yielded erbB2 gene amplification by *FISH* (Fig. 3). The results of immunoprecipitation experiments and immunofluorescent stainings revealed a direct interaction between MUC4 and erbB2 in the carcinoma tissues (Fig. 1) and their partial co-localisation in the cancerous epithelia (Fig. 2). With the interaction with EGFR, erbB3 or erbB4, a previous study reported that no interactions were found in the transfection experiments.[17]

With respect to pathological significance, it should be noted that there was a strong correlation between the histological grade (well and moderately differentiated adenocarcinomas) and the expression levels of MUC4, and that the co-expression of MUC4 and erbB2 was present in about one-fourth of the cases of well and moderately differentiated adenocarcinomas. However, no other significant differences in the expression rates of MUC4 and erbB2 were found with respect to histological grade, distant organ metastasis, pathological features and survival outcome of the patients (Table 2 and Fig. 3). Both the mRNA levels and the expression rates of MUC4 tended to be higher in the cases of pT1 and pT2 carcinomas than in those of pT3 and pT4 carcinomas (Fig. 3 and Table 2). Furthermore, both the mRNA levels and the expression rates were significantly lower in the cases of poorly differentiated carcinoma, the more aggressive type of the disease, than in those of well or moderately differentiated

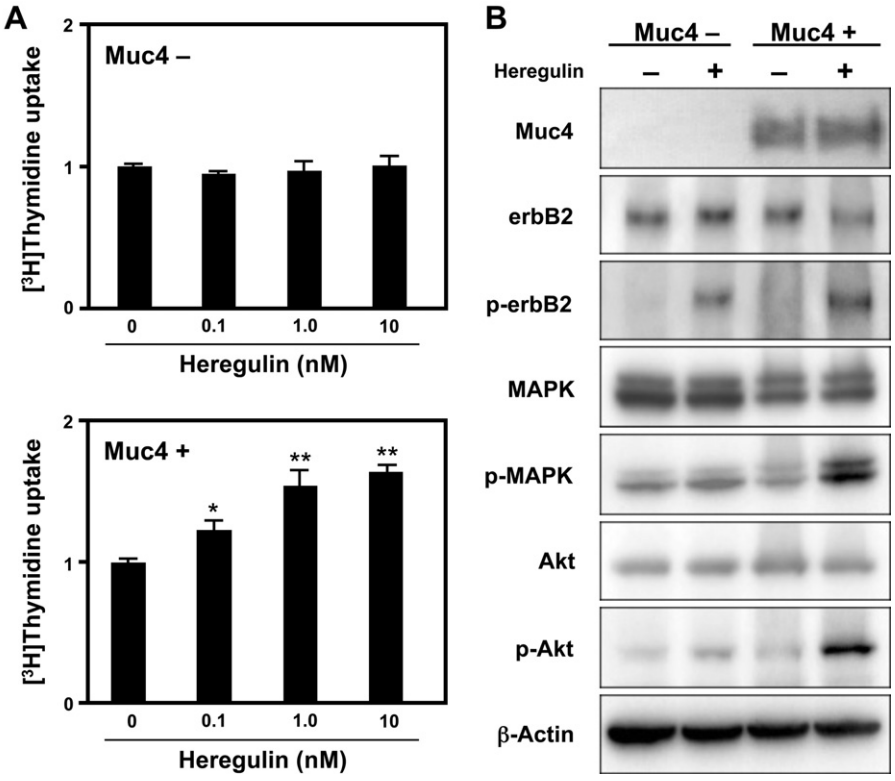

Fig. 6 – Biological effects of Muc4 on erbB2 signalling and proliferating activity *in vitro*. Effect of Muc4 on [^{3}H]-thymidine uptake (A) and expression status of erbB2, MAPK and Akt (B) in heregulin-stimulated A375 cells. *P < 0.05, **P < 0.01, different from the vehicle-treated cells.

carcinoma (Fig. 3 and Table 2). Combining the above results with the observation that Muc4 is up-regulated in the process of gallbladder carcinogenesis in BK5.erbB2 mice[13], MUC4 may play an important role in the carcinogenesis rather than the progression of well and moderately differentiated types of human gallbladder carcinomas.

On examination of erbB2 downstream signalling pathways, hyperphosphorylation of MAPK and Akt was observed in human gallbladder carcinoma tissues (Fig. 5). Hyperphosphorylation of MAPK and Akt was also observed in gallbladders of BK5.erbB2 mice that overexpress activated erbB2 (data not shown), suggesting that MAPK and/or PI3K signalling pathways may play a role in the development of gallbladder carcinoma. Moreover, the results of Muc4 transfectant cell experiments suggest that the presence of Muc4 potentiates the effects of heregulin on the phosphorylation of erbB2, MAPK and Akt, which in turn enhances the tumour growth (Fig. 6).

Supporting the results of this study, in rat cholangiocyte transformants overexpressing activated erbB2/neu[26], an enhanced downstream signalling was observed to be p44/42MAPK and p60 Akt. A selective inhibitor of erbB2 tyrosine kinase exerts a potent antitumour activity through suppressing the activation of Akt, an anti-apototic molecule, in erbB2-positive breast carcinoma cells but not in erbB2-negative cells.[27]

In summary, the findings obtained in human gallbladder carcinoma suggest that the up-regulation of MUC4 and its interaction with erbB2 play an important role in gallbladder carcinogenesis through potentiating the receptor tyrosine kinase and alteration of erbB2 signalling in gallbladder epithelia. Transgenic approaches would be invaluable in similar situations and undoubtedly prove helpful in further elucidating the biological roles of Muc4/MUC4 in biliary tract carcinogenesis. Therapeutic options for gallbladder carcinoma are still limited. Based on the results of this study, targeting MUC4 and erbB2 could provide a new and effective therapy for a number of patients with gallbladder carcinoma.

Conflict of interest statement

None declared.

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