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ORIGINAL ARTICLE

# DDR2 and IFITM1 Are Prognostic Markers in Gallbladder Squamous Cell/Adenosquamous Carcinomas and Adenocarcinomas

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**Abstract** This study was conducted to investigate the expressions of DDR2 and IFITM1 and their clinical and pathological significances in the rare type squamous cell/adenosquamous carcinomas (SC/ASC) and ordinary adenocarcinomas (AC) of gallbladder cancers. DDR2 and IFITM1 expression was examined in 69 SC/ASCs and 146 ACs using EnVision immunohistochemistry. Results showed that the percentage of positive DDR2 and IFITM1 expression was significantly higher in SC/ASC patients with high TNM stage, lymph node metastasis, invasion, and no resection surgery compared to patients with low TNM stages, no lymph node metastasis, no invasion, and resection surgery ($P < 0.05$ or $P < 0.01$). The positive rate of DDR2 was significantly higher in SC/ASC patients with large tumor sizes than patients with small tumor sizes ($p < 0.05$). The percentage of positive DDR2 and IFITM1 expressions was significantly higher in AC patients with high TNM stages that didn't receive resection surgery compared to patients with low TNM stages that did receive resection surgery ($P < 0.05$ or $P < 0.01$). The positive rate of IFITM1 was significantly higher in AC patients with lymph node metastasis and invasion than in patients without metastasis and invasion ($p < 0.05$). Positive DDR2 and IFITM1 expression was closely associated with a decreased overall survival in SC/ASC and AC patients ($P < 0.05$ or $P < 0.01$). AUC analysis showed that DDR2 and IFITM1 was sensitive and specific for the diagnosis of SC/ASC (AUC = 0.740 and AUC =0.733, respectively) and AC (AUC = 0.710 and AUC =0.741, respectively). In conclusion, positive DDR2 and IFITM1 expression is a marker for the clinical severity, poor prognosis, and diagnosis of gallbladder SC/ASC and AC.

**Keywords** Gallbladder · Squamous cell carcinoma · Adenosquamous carcinomas · DDR2 · IFITM 1 · Immunohistochemistry

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## Introduction

Gallbladder cancers (GBCs) are relatively uncommon, but highly lethal diseases with a particular geographical distribution worldwide [1, 2]. Most GBCs are adenocarcinomas (AC >90%) [3], followed by squamous cell/adenosquamous carcinoma (SC/ASC) (accounting for 1–6% of GBCs) [2, 4]. Early diagnosis of GBC is difficult because early stage GBC is commonly asymptomatic [5]. Approximately 50% of GBC patients were diagnosed incidentally following a cholecystectomy [6]. More than 90% of GBC patients are diagnosed at an inoperable stage with invasive and metastatic tumors [7]. A series study in France reported that 85% of T3/T4 GBC tumors only had a median survival of 3–6 months [8]. In contrast, the 5-year survival following for resected T1a gallbladder cancer is about 97–99% [9]. The 5-year survival for GBC patients with T2 tumors varies depending on the surgery (17–38% for simple cholecystectomy and 59–90% extended cholecystectomy) [10, 11]. A meta-analysis for the adjuvant chemotherapy, radiotherapy, or both in GBCs demonstrated a nonsignificant improvement in survival comparing any adjuvant therapy with surgery alone [12]. Targeted therapies against epidermal growth factor receptor (EGFR) and vascular

endothelial growth factor (VEGF) showed no or only marginal benefits on the survival of GBC patients [13]. Therefore, it is crucial to understand the carcinogenesis and key molecular pathways of GBCs to establish effective targeted therapies.

Extracellular matrix (ECM) is a complex extracellular structural system that provides structural and biochemical support to the cells [14]. The matrix metalloproteinases (MMPs) are enzymes responsible for the collagen and other protein degradation in ECM [15]. MMPs play an important role in malignant tumor growth, cancer cell survival, and metastasis [16]. Discoidin domain receptor 2 (DDR2) belongs to the receptor tyrosine kinase (RTK) family and is activated by collagen binding [17]. The main functions of DDR2 are to control the remodeling of ECM by regulating the expression and activity of MMPs [18], and promote cell proliferation, cell adhesion, and cell migration [19]. In tumors, DDR2 participates in the process of melanoma liver metastasis [20], and colorectal cancer metastasis [21]. High DDR2 expression has been reported to be associated with the fast progression and poor prognosis of breast cancer [22], non-small cell lung cancer [23], hepatocellular cancer [24], gastric cancer [25], prostate cancer [26], bladder cancer [27], and ovarian cancer [28].

Interferon induced transmembrane protein 1 (IFITM1) was initially identified as a leukocyte antigen and as a part of a

**Table 1** Comparison of gallbladder SC/ASC and AC clinicopathological features and DDR2 and IFITM1 expression status

| Clinicopathological characteristics | SC/ASC ($n$ = 69) | AC ($n$ = 146 | $\chi^2$ | $P$ value |
|---|---|---|---|---|
| Gender, n (%) | | | | |
| Male | 25(36.2) | 61(41.8) | 0.601 | 0.438 |
| Female | 44(63.8) | 85(58.2) | | |
| Age, n (%) | | | | |
| ≤ 45 years | 3(4.3) | 20(13.7) | 4.289 | 0.038 |
| > 45 years | 66(95.7) | 126(86.3) | | |
| Differentiation, n (%) | | | | |
| Well | 19(27.5) | 51(34.9) | 2.235 | 0.308 |
| Moderate | 33(47.8) | 54(37.0) | | |
| Poor | 17(24.6) | 41(28.1) | | |
| Maximum tumor diameter, n (%) | | | | |
| ≤ 3 | 39(56.5) | 90(61.6) | 0.512 | 0.474 |
| > 3 cm | 30(43.5) | 56(38.4) | | |
| Cholecystolithiasis, n (%) | | | | |
| no | 31(44.9) | 78(53.4) | 1.353 | 0.245 |
| yes | 38(55.1) | 68(46.6) | | |
| TNM stages, n (%) | | | | |
| I + II | 29(42.0) | 77(52.7) | 2.151 | 0.143 |
| III + IV | 40(58.0) | 69(47.3) | | |
| Lymph node metastasis, n (%) | | | | |
| no | 27(39.1) | 80(54.8) | 4.599 | 0.032 |
| yes | 42(60.9) | 66(45.2) | | |
| Invasion, n (%) | | | | |
| no | 24(34.8) | 72(49.3) | 4.004 | 0.045 |
| yes | 45(65.2) | 74(50.7) | | |
| Surgical methods, n (%) | | | | |
| Radical | 27(39.1) | 75(51.4) | 3.002 | 0.223 |
| Palliative | 28(40.6) | 50(34.2) | | |
| Biopsy | 14(20.3) | 21(14.4) | | |
| DDR2 | | | | |
| - | 29(42.0) | 65(44.5) | 0.660 | 0.417 |
| + | 40(58.0) | 70(47.9) | | |
| IFITM1 | | | | |
| - | 30(43.5) | 61(41.8) | 0.055 | 0.814 |
| + | 39(56.5) | 85(58.2) | | |

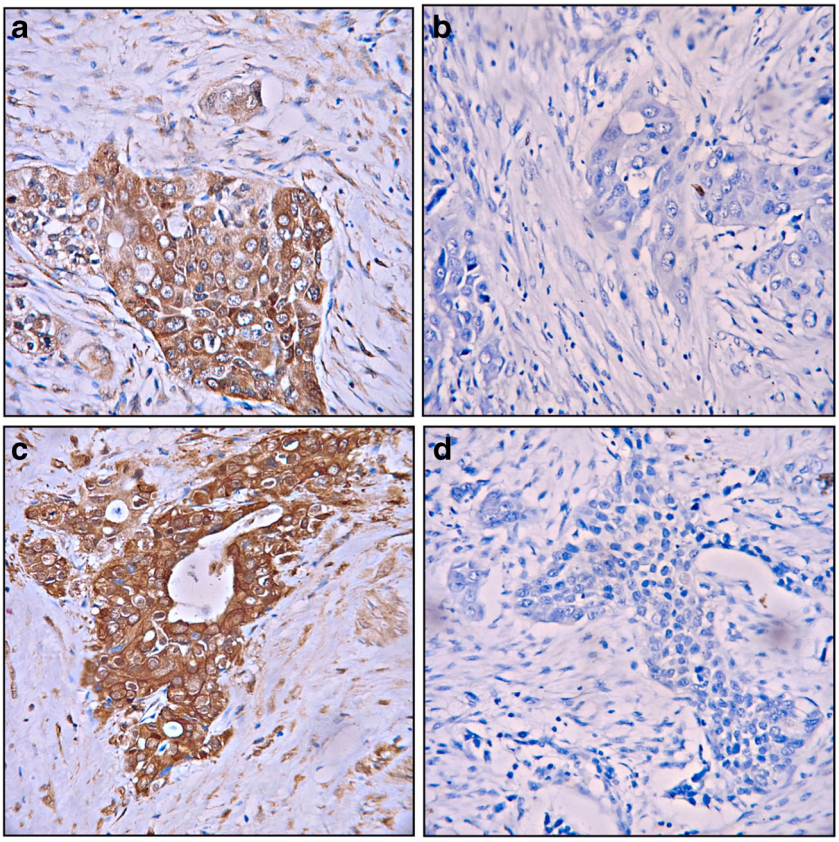


**Fig. 1** DDR2 and IFITM1 expression in SC/ASC tumors. DDR2 and IFITM1 expression was localized in the cytoplasm. **a** Positive DDR2 expression in moderately differentiated SC. **b** Negative DDR2 expression in well differentiated SC. **c** Positive IFITM1 expression in in moderately differentiated ASC. **d** Negative IFITM1 expression in moderately differentiated SC. Original magnification ×200

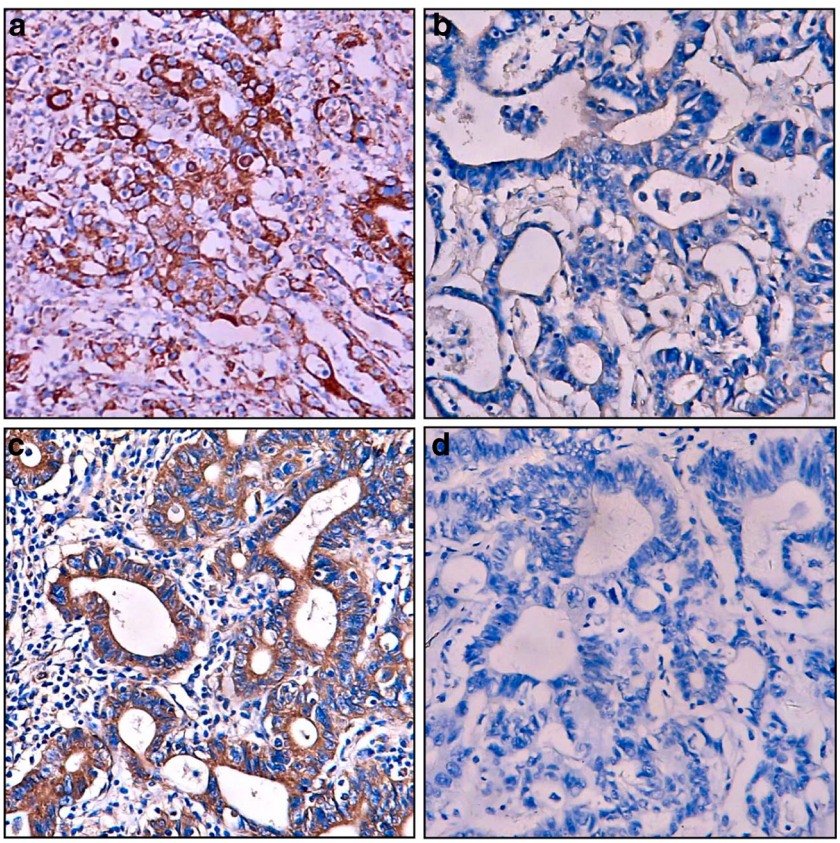


**Fig. 2** DDR2 and IFITM1 expression in AC tumors. DDR2 and IFITM1 expression was localized in the cytoplasm. **a** Positive DDR2 expression in poorly differentiated adenocarcinoma. **b** Negative DDR2 expression in well differentiated adenocarcinoma. **c** Positive IFITM1 expression in well differentiated adenocarcinoma. **d** Negative IFITM1 expression in moderately differentiated adenocarcinoma. Original magnification ×200

membrane complex involved in the transduction of antiproliferative and homotypic cell adhesion signals in lymphocytes [29]. IFITM1 could be highly induced by IFN-α and IFN-γ in response to pathogens [29]. Most recent studies suggest that IFITM1 might play a role in tumorigenesis. Overexpression of IFITM1 was found in breast cancer cases [30], esophageal and gastric cancer cases [31], colorectal cancer cases [32] and ovarian cancer cases [33]. Suppression of IFITM1 expression could inhibit the proliferation and invasion of glioma cells [34]. The roles of IFITM1 in cancer may be associated with its regulatory effects on MMPs. For example, Kim et al. study demonstrated that IFITM1 can induce the expression of MMP-9 [35]. Deraz et al. suggested that IFITM1 can upregulate MMP-10 expression and subsequently promote the invasion and metastasis of HNSCC cells [36]. He et al. study revealed that IFITM1 can enhance the proliferation, invasion, and metastasis of colorectal cancer cells [37].

The expression of DDR2 and IFITM1 in gallbladder SC/ASC and AC has not been reported. This study investigated DDR2 and IFITM1 expression in 146 ACs and 69 SC/ASCs using immunohistochemistry and their association with the clinicopathological and biological characteristics of AC and SC/ASC.

## Materials and Methods

### Case Selection

A total of 69 SC/ASC tumor tissues and 146 AC tumor tissues were collected during surgical resection or biopsy from January 2001 to December 2013. The subtypes of GBC were diagnosed according to the recommendations of the American Joint Committee on Cancer. The tumor tissues were routinely paraffin-embedded. The 69 SC/ASC patients included 47 females (F/M = 2.14) with an age range of 35 to 80 (53.8 ± 10.2) years old, while the 146 AC patients included 92 females (F/M = 1.77) with an age range of 33 to 78 (52.4 ± 9.6) years old. 19 (27.5%), 33 (47.8%), and 17 (24.6%) of the 69 SC/ASCs were well, moderately, and poorly differentiated, respectively. 51 (34.9%), 54 (37.0%), and 41 (28.1%) of the 146 ACs were well, moderately, and poorly differentiated, respectively. Invasion of tissues and organs surrounding the gallbladder was observed in 45 (65.2%) SC/ASC patients and 74 (50.7%) AC patients. 42 (60.7%) SC/ASC patients and 66 (45.2%) AC patients had regional lymph node metastasis. 38 (55.1%) SC/ASC patients and 68 (46.6%) patients had gallstones. 29 of the 69 SC/ASCs were tumor-node-metastasis (TNM) stage I + II, and 40 were TNM stage III+ IV. 77 of

**Table 2** Correlations of DDR2 and IFITM1 protein expression with the clinicopathological characteristics of gallbladder SC/ASC

| CPC | Case No. | DDR2 | | | IFITM1 | | |
|---|---|---|---|---|---|---|---|
| | | Pos No. (%) | $\chi^2$ | P value | Pos No. (%) | $\chi^2$ | P value |
| Differentiation | | | | | | | |
| Well | 19 | 8(42.1) | 4.353 | 0.113 | 10(52.6) | 0.633 | 0.729 |
| Moderately | 33 | 19(57.6) | | | 18(54.5) | | |
| Poorly | 17 | 13(76.5) | | | 11(64.7) | | |
| Tumor size | | | | | | | |
| ≤ 3 cm | 30 | 13(43.3) | 4.668 | 0.031 | 14(46.7) | 2.098 | 0.148 |
| > 3 cm | 39 | 27(69.2) | | | 25(64.1) | | |
| Gallstone | | | | | | | |
| No | 31 | 19(61.3) | 0.255 | 0.614 | 19(61.3) | 0.521 | 0.470 |
| Yes | 38 | 21(55.3) | | | 20(52.6) | | |
| Lymph node metastasis | | | | | | | |
| No | 27 | 11(40.7) | 5.405 | 0.020 | 11(40.7) | 4.495 | 0.034 |
| Yes | 42 | 29(69.0) | | | 28(66.7) | | |
| Invasion | | | | | | | |
| No | 24 | 8(33.3) | 9.168 | 0.002 | 8(33.3) | 8.052 | 0.005 |
| Yes | 45 | 32(71.1) | | | 31(68.9) | | |
| TNM stage | | | | | | | |
| I + II | 29 | 12(41.4) | 5.652 | 0.017 | 12(41.4) | 4.668 | 0.031 |
| III + IV | 40 | 28(70.0) | | | 27(67.5) | | |
| Surgery | | | | | | | |
| Radical | 27 | 9(33.3) | 12.273 | 0.002 | 10(37.0) | 6.901 | 0.032 |
| Palliative | 28 | 19(67.9) | | | 19(67.9) | | |
| Biopsy | 14 | 12(85.7) | | | 10(71.4) | | |

the 146 AC patients were TNM stage I + II, and 69 were TNM stage III+ IV. Surgery included radical resection for 27 SC/ASCs and 75 ACs, palliative surgery for 28 SC/ASCs and 50 ACs, and no operation for 14 SC/ASCs and 21 ACs which were only biopsied (Table 1). Survival data were obtained from all patients. The follow-up time was 2 years, and patients who survived longer than 2 years were included in the analysis as censored cases. Of the 146 AC patients, 58 survived more than 1 year (26 more than 2 years), and 88 survived <1 year. Of the 46 SC/ASC patients, 17 survived more than 1 year (7 more than 2 years), and 52 survived <1 year.

### EnVision Immunohistochemistry

The rabbit anti-human DDR2 and IFITM1 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). EnVision™ Detection Kit was purchased from Dako Laboratories (CA, USA). The staining of DDR2 and IFITM1 was carried out according to the manufacture's protocol. Briefly, the paraffin-embedded tumor tissues were sectioned at 4-μM thick and deparaffinized. After incubating with peroxidase inhibitor (3% $H_2O_2$) for 15 min and heat-induced epitope retrieval with sodium citrate buffer for 20 min at 98 °C, the sections were incubated with primary antibody for 120 min, followed by incubation with Solution A (containing HRP-conjugated secondary antibody) for 30 min, DAB staining, and hematoxylin counter-staining. After being dehydrated with 70%–100% alcohol, sections were soaked in xylene for 3 × 5 min, followed by mounting with neutral balsam. Ten random fields per section were viewed and the percent of positively stained cells relative to the total number of cells in each section was determined, and the average percentage per case was calculated from 5 sections. At the same time, the strength of staining was rated on a scale of 1 to 3. A score of 1 represented no positive staining or uncertainly weak staining; a score of 2 represented weak to moderate staining; and a score of 3 represented moderate to strong staining. A case was determined as positive DDR2 or IFITM1 when the average percentage of positively stained cells was ≥10% and staining strength ≥2. The few cases whose percentage of positive staining was 5% to 10% and staining strength was 3 were also regarded as positive.

### Statistical Analysis

Data were analyzed using the statistical package for the Social Sciences Version 13.0 (SPSS 13.0). The inter-relationship of DDR2 or IFITM1 expression with histology or clinical factors was determined using $\chi 2$ or Fisher's exact test. Univariate survival analysis was performed by Kaplan-Meier and time

**Table 3** Correlations of DDR2 and IFITM1 protein expression with the clinicopathological characteristics of gallbladder AC

| CPC | Case No. | DDR2 | | | IFITM1 | | |
|---|---|---|---|---|---|---|---|
| | | Pos No. (%) | $\chi^2$ | P value | Pos No. (%) | $\chi^2$ | P value |
| Differentiation | | | | | | | |
| Well | 51 | 27(52.9) | 4.073 | 0.130 | 27(52.9) | 3.676 | 0.159 |
| Moderately | 54 | 23(42.6) | | | 29(53.7) | | |
| Poorly | 41 | 26(63.4) | | | 29(70.7) | | |
| Tumor size | | | | | | | |
| ≤ 3 cm | 90 | 46(51.1) | 0.084 | 0.772 | 48(53.3) | 2.303 | 0.129 |
| > 3 cm | 56 | 30(53.6) | | | 37(66.1) | | |
| Gallstone | | | | | | | |
| No | 78 | 45(57.7) | 2.133 | 0.144 | 50 (64.1) | 2.383 | 0.123 |
| Yes | 68 | 31(45.6) | | | 35(51.5) | | |
| Lymph node metastasis | | | | | | | |
| No | 80 | 37(46.3) | 2.389 | 0.122 | 39(48.8) | 6.523 | 0.011 |
| Yes | 66 | 39(59.1) | | | 46(69.7) | | |
| Invasion | | | | | | | |
| No | 72 | 33(45.8) | 2.203 | 0.138 | 36(50.0) | 3.945 | 0.047 |
| Yes | 74 | 43(58.1) | | | 49(66.2) | | |
| TNM stage | | | | | | | |
| I + II | 77 | 37(48.1) | 1.046 | 0.306 | 35(45.5) | 10.914 | 0.001 |
| III + IV | 69 | 39(56.6) | | | 50(72.5) | | |
| Surgery | | | | | | | |
| Radical | 75 | 37(49.3) | 3.710 | 0.156 | 37(49.3) | 9.026 | 0.011 |
| Palliative | 50 | 24(48.0) | | | 30(60.0) | | |
| Biopsy | 21 | 15(71.4) | | | 18(85.7) | | |

series test. Multivariate analysis was conducted with a Cox proportional hazards model. The receiver operating characteristic (ROC) curves were constructed with sensitivity being x and 1-specificity being y. The AUC of the ROC were calculated for evaluating the overall diagnostic performance.

### Ethics

The use of patients' tumor tissues and clinical data was pre-approved by the Ethics Committee for Human Research of Central South University and local hospitals.

## Results

### DDR2 and IFITM1 Expression and Clinicopathological Characteristics in SC/ASC and AC

The percentage of cases with an age of >45 years, lymph node metastasis, and invasion was significantly higher in the SCs/ASCs compared with the ACs ($P < 0.05$). No significant correlations were observed between the percentage of positive DDR2 or IFITM1 expression and other clinicopathological characteristics. The EnVision immunohistochemistry showed that the majority of DDR2- and IFITM1-positive reactions were localized in the cytoplasm of both the SC/ASCs (Fig. 1) and ACs (Fig. 2).

### Positive DDR2 and IFITM1 Expression Was Associated with the Clinicopathological Characteristics in SC/ASC and AC Patients

The percentage of positive DDR2 and IFITM1 expression was significantly higher in SC/ASC with high TNM stage, lymph node metastasis, invasion, and no resection (biopsy only) compared to SC/ASC patients with low TNM stage, no lymph node metastasis, no invasion, and no radical resection ($P < 0.01$; Table 2). The positive rate of DDR2 was significantly higher in SC/ASC patients with large tumor sizes than SC/ASC patients with small tumor sizes ($p < 0.05$; Table 2).

In AC patients, the percentage of positive DDR2 and IFITM1 expression was significantly higher in the cases with

**Table 4** Relationships between DDR2 and IFITM1 expression, clinicopathological characteristics and average survival of SC/ASC patients

| Clinicopathological characteristics | Sample (n) | Average survival (month) | $\chi^2$ | P value |
|---|---|---|---|---|
| Differentiation | | | | |
| Well | 19 | 13.68(5–24) | 20.815 | 0.000 |
| Moderately | 33 | 11.58(4–24) | | |
| Poorly | 17 | 6.12(2–14) | | |
| Tumor size | | | | |
| ≤ 3 cm | 30 | 14.57(6–24) | 21.493 | 0.000 |
| > 3 cm | 39 | 7.44(2–24) | | |
| Gallstones | | | | |
| No | 31 | 8.26(3–18) | 7.125 | 0.008 |
| Yes | 38 | 12.90(2–24) | | |
| TNM stage | | | | |
| I + II | 29 | 16.31(3–24) | 46.137 | 0.000 |
| III + IV | 40 | 6.83(2–14) | | |
| Lymph node metastasis | | | | |
| No | 27 | 16.04(3–24) | 29.663 | 0.000 |
| Yes | 42 | 7.45(2–15) | | |
| Invasion | | | | |
| No | 24 | 17.25(3–24) | 36.974 | 0.000 |
| Yes | 45 | 7.38(2–20) | | |
| Surgery | | | | |
| Radical | 27 | 16.93(5–24) | 54.660 | 0.000 |
| Palliative | 28 | 7.32(2–12) | | |
| Biopsy | 14 | 6.00(4–8) | | |
| DDR2 | | | | |
| - | 29 | 14.62(3–24) | 8.890 | 0.003 |
| + | 40 | 8.45(2–24) | | |
| IFITM1 | | | | |
| - | 30 | 15.13(3–24) | 10.827 | 0.001 |
| + | 39 | 8.36(2–20) | | |

high TNM stage and no resection than in cases with low TNM stage and radical resection ($P < 0.05$ or $P < 0.01$; Table 3). The positive rate of IFITM1 was significantly higher in AC patients with lymph node metastasis and invasion than AC patients without lymph node metastasis and invasion ($p < 0.05$; Table 3).

Thirty-one of the 40 DDR2 positive SC/ASC cases were IFITM1 positive, while 21 of the 29 DDR2 negative SC/ASC cases were IFITM1 negative, suggesting a high consistency between these two markers in SC/ASC patients ($\chi2 = 17.043$, $p < 0.05$). 52 of the 76 DDR2 positive AC cases were IFITM1 positive, while 37 of the 70 DDR2 negative AC cases were IFITM1 negative, suggesting a consistency between these two markers in AC patients ($\chi2 = 6.269$, $p < 0.05$).

## Positive DDR2 and IFITM1 Expression Correlate with Lower Survival Rates in Patients with SC/ASC and AC

Kaplan-Meier survival analysis showed that poor tumor differentiation, larger tumor size, high TNM stage, lymph node metastasis, invasion, no resection surgery, gallstone, and positive DDR2 and IFITM1 expressions were significantly associated with shorter overall survival time in SC/ASC patients (P<0.01, Table 4, Fig. 3a, b). Cox's multivariate analysis showed that differentiation, tumor size, TNM stage, invasion, surgical procedure, and DDR2- and IFITM1-positive expressions were risk factors of SC/ASC (Table 5).

Kaplan-Meier survival analysis showed similar outcomes between AC and SC/ASC patients (Table 6). AC patients with DDR2 and IFITM1 expressions exhibited a significantly lower survival rate than AC patients with negative DDR2 and IFITM1 expressions (P<0.01; Table 6 and Fig. 3c, d). Cox's multivariate analysis showed that differentiation, tumor size, TNM stage, lymph node metastasis, invasion, surgical procedure, and DDR2 and IFITM1 positive expression were risk factors of the AC patients (Table 7).

## DDR2 and IFITM1 Exhibited a Better Diagnostic Performance for SC/ASC and AC

The AUC of DDR2 was 0.740 (95% CI: 0.640–0.840) (Fig. 4a), while the AUC of IFITM1 was 0.733 (95% CI: 0.631–0.834) (Fig. 4b) in SC/ASC. The AUC of DDR2 was 0.710 (95% CI: 0.621–0.799) (Fig. 4c) and the AUC of IFITM1 was 0.741 (95% CI: 0.655–0.827) (Fig. 4d) in AC. These findings suggest that both DDR2 and IFITM1 had a better overall diagnostic performance.

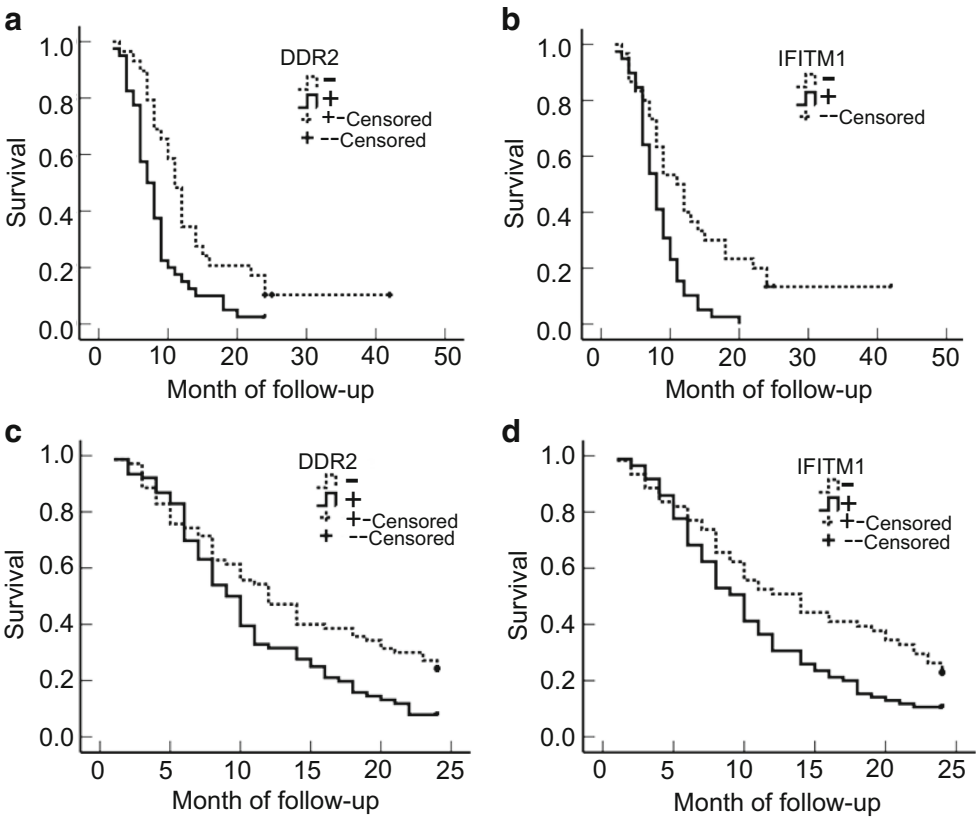


**Fig. 3** DDR2 and IFITM1 expression and survival in patients with SC/ASC and AC cancer. **a** Overall survival curve in SC/ASC patients with DDR2-positive and DDR2–negative expression. **b** Overall survival curve in SC/ASC patients with IFITM1-positive and IFITM1-negative expression. **c** Overall survival curve in AC patients with DDR2-positive and DDR2–negative expression. **d** Overall survival in AC patients with IFITM1-positive and IFITM1-negative expression

**Table 5** Multivariate Cox regression analysis of survival rate in SC/ASC patients

| Groups | Factors | B | SE | wald | *P* | RR | 95% CI | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower | Upper |
| Differentiated degree | well/moderately/poorly | .561 | .215 | 6.828 | .009 | 1.753 | 1.151 | 2.671 |
| Tumor size | ≤3 cm/>3 cm | .758 | .366 | 4.295 | .038 | 2.134 | 1.042 | 4.372 |
| Gallstone | No/yes | .617 | .289 | 4.563 | .033 | 1.853 | 1.052 | 3.262 |
| TNM stage | I + II/III + IV | .961 | .442 | 4.732 | .030 | 2.613 | 1.100 | 6.209 |
| Lymph node metastasis | No/yes | 1.147 | .439 | 6.813 | .009 | 3.148 | 1.331 | 7.447 |
| Invasion | No/yes | 1.487 | .571 | 6.780 | .009 | 4.424 | 1.444 | 13.552 |
| Surgery | radical/palliative/biopsy | .801 | .290 | 7.609 | .006 | 2.228 | 1.261 | 3.936 |
| DDR2 | −/+ | .857 | .297 | 8.356 | .004 | 2.357 | 1.318 | 4.214 |
| IFITM1 | −/+ | 1.030 | .311 | 11.002 | .001 | 2.801 | 1.524 | 5.148 |

## Discussion

The matrix metalloproteinases mediated degradation of collagen and other protein in the extracellular matrix of tumor cells is an important step for tumor cell metastasis [a7]. DDR2 and IFITM1 can regulate the expression and activity of matrix metalloproteinases and subsequently is involved in the tumorigenesis, invasion, and metastasis of tumors [18, 35–37]. However, although their expressions have been associated with the progression and prognosis of a variety of tumors,

**Table 6** Relationships between DDR2 and IFITM1 expression, clinicopathological characteristics and overall survival of AC patients

| Clinicopathological characteristics | Sample (n) | Average survival (month) | $\chi^2$ | P value |
|---|---|---|---|---|
| Differentiation | | | | |
| Well | 51 | 16.69(5–24) | 55.112 | 0.000 |
| Moderately | 54 | 12.33(2–24) | | |
| Poorly | 41 | 6.49(1–24) | | |
| Tumor size | | | | |
| ≤ 3 cm | 90 | 14.60(1–24) | 23.174 | 0.000 |
| > 3 cm | 56 | 8.38(1–24) | | |
| Gallstones | | | | |
| No | 78 | 12.19(2–24) | 0.001 | 0.980 |
| Yes | 68 | 12.24(1–24) | | |
| TNM stage | | | | |
| I + II | 77 | 16.99(3–24) | 87.485 | 0.000 |
| III + IV | 69 | 6.88(1–24) | | |
| Lymph node metastasis | | | | |
| No | 80 | 16.35(2–24) | 71.402 | 0.000 |
| Yes | 66 | 7.20(1–24) | | |
| Invasion | | | | |
| No | 72 | 18.08(4–24) | 124.522 | 0.000 |
| Yes | 74 | 6.50(1–14) | | |
| Surgery | | | | |
| Radical | 75 | 17.84(6–24) | 150.255 | 0.000 |
| Palliative | 50 | 6.86(1–14) | | |
| Biopsy | 21 | 4.86(1–9) | | |
| DDR2 | | | | |
| - | 70 | 16.63(1–24) | 6.955 | 0.008 |
| + | 76 | 10.91(1–24) | | |
| IFITM1 | | | | |
| - | 61 | 14.05(1–24) | 6.318 | 0.012 |
| + | 85 | 10.89(1–24) | | |

**Table 7** Multivariate Cox regression analysis of survival rate in AC patients

| Groups | Factors | B | SE | wald | *P* | RR | 95% CI | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower | Upper |
| Differentiated degree | Well/moderately/poorly | .558 | .167 | 1.183 | .001 | 1.748 | 1.260 | 2.424 |
| Tumor size | ≤3 cm/>3 cm | .739 | .351 | 4.419 | .036 | 2.093 | 1.051 | 4.167 |
| Gallstone | No/yes | .235 | .214 | 1.206 | .272 | 1.265 | .832 | 1.924 |
| TNM stage | I + II/III + IV | .906 | .430 | 4.439 | .035 | 2.474 | 1.065 | 5.744 |
| Lymph node metastasis | No/yes | .923 | .354 | 6.804 | .009 | 2.516 | 1.258 | 5.032 |
| Invasion | No/yes | 1.882 | .445 | 17.932 | .000 | 6.570 | 2.749 | 15.702 |
| Surgery | Radical/ Palliative/ Biopsy | .808 | .270 | 8.915 | .003 | 2.243 | 1.320 | 3.811 |
| DDR2 | −/+ | .749 | .245 | 9.319 | .002 | 2.115 | 1.308 | 3.422 |
| IFITM1 | −/+ | .664 | .262 | 6.418 | .011 | 1.942 | 1.162 | 3.244 |

DDR2 and IFITM1 expressions and their significance in GBC have not been reported. This study investigated the immunohistochemical staining of DDR2 and IFITM1 in 146 AC and 69 SC/ASC. A significant increase in DDR2 and IFITM1 expressions in AC and SC/ASC tumors was observed. Positive DDR2 and IFITM1 expressions are associated with TNM stages, invasion, metastasis, and poor prognosis of AC and SC/ASC.

The AC is an ordinary type of GBC (over 90%) whereas SC/ASCs are rare types of GBC (1–12%) [2–4]. The current knowledge on the clinicopathological characteristics of SC/ASC has mainly been obtained from individual case studies

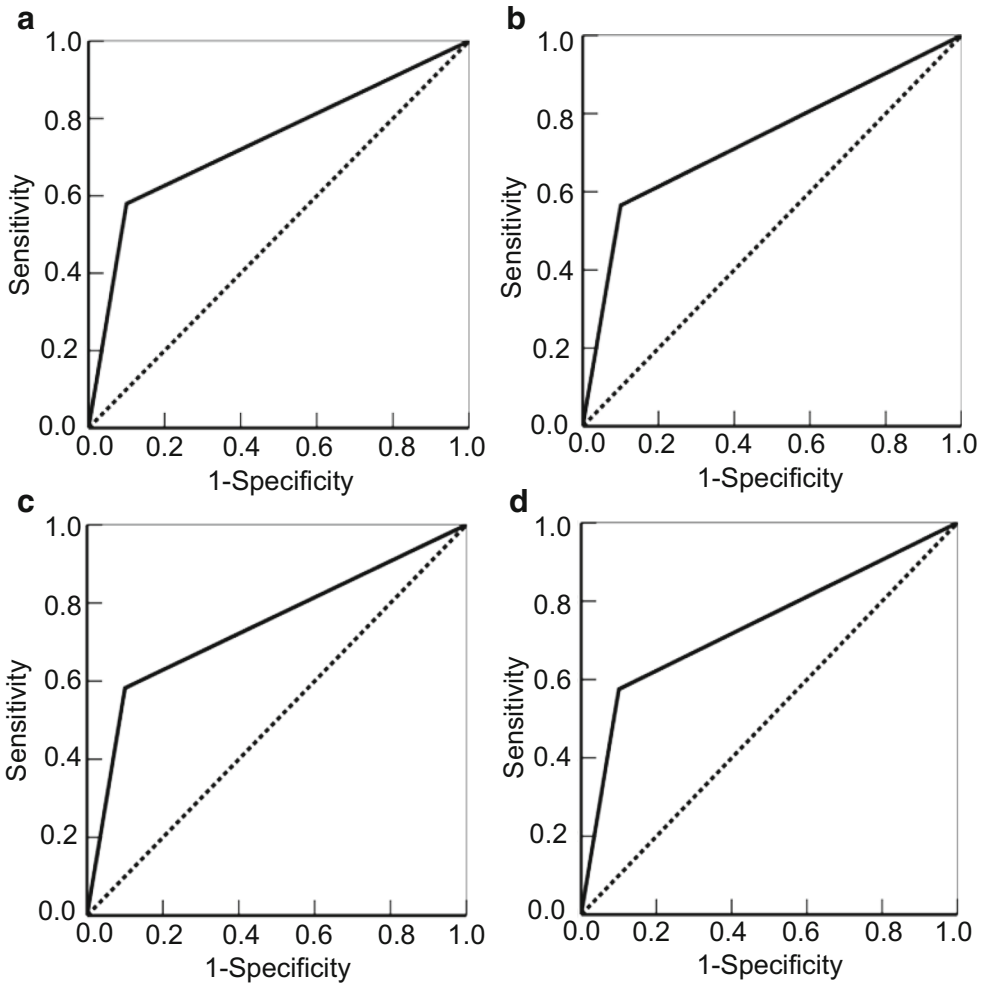


**Fig. 4** ROC curves. ROC of diagonal segments was produced by ties of DDR2 (**a**) in SC/ASC, IFITM1 (**b**) in SC/ASC, DDR2 (**c**) in AC, and IFITM1 (**d**) in AC

or analyses of small case series. 69 cases are the largest SC/ASC samples in current reports. Therefore, it can provide us more opportunity to accurately understand the differences between rare SC/ASC tumors and ordinary adenocarcinomas. Previous opinions thought that squamous tumors had higher proliferation rates than adenocarcinomas, but lymph node metastasis occurs less in squamous tumors than in adenocarcinomas [38, 39]. In contrast, the present study revealed that the percentage of cases with lymph node metastasis and invasion was significantly higher in the SCs/ASCs compared to the ACs ($P < 0.05$). However, there were no significant differences in the differentiation degrees, tumor sizes, TNM stages, surgical methods, DDR2 and IFITM1 expressions, and overall survival between AC and SC/ASC patients. This study suggests that the rare SC/ASC and ordinary AC have similar clinical, pathological, and biological characteristics.

The discoidin domain receptors, DDR1 and DDR2, are two closely related receptor tyrosine kinases (RTKs); they are characterized by structurally diverse extracellular ligand-binding regions and conserved cytosolic kinase domains. RTK-dependent cellular signaling controls critical cellular processes, including proliferation, differentiation, cell survival, cell migration, and cell cycle control [40]. Overexpression of DDR2 has been reported in a variety of tumors and is associated with metastasis and poor prognosis [20–28]. IFITM1 is a member of the interferon-induced transmembrane protein family and is induced by interferon $\alpha$ and $\gamma$ to involve in interferon-mediated antiviral, anti-inflammation, and anti-proliferation activities in immune system [41]. Also, IFITM1 has been demonstrated to promote cancer progression by enhancing cell migration and invasion in gastric cancer and head and neck cancer [42]. The roles of DDR2 and IFITM1 in tumors may be associated with their regulatory function on the expression and activity of matrix metalloproteinases. Our study first revealed that the positive DDR2 and IFITM1 expressions were significantly higher in SC/ASC and AC patients with large tumor sizes, a high TNM stage, lymph node metastasis, invasion and no resection surgery. Also, positive DDR2 and IFITM1 expressions are independent factors for a poor-prognosis in SC/ASC and AC patients and can be used for the diagnosis of GBC.

In conclusion, DDR2 and IFITM1 are involved in the progression of SC/ASC and AC, and positive DDR2 and IFITM1 expressions are associated with poor prognosis in patients with SC/ASC and AC.

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