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ORIGINAL ARTICLE

# Expression and prognostic value of HER-2/neu, STAT3 and SOCS3 in hepatocellular carcinoma

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**KEYWORDS**
Primary hepatocellular carcinoma;
Human epidermal growth factor receptor-2;
Signal transducer and activator of transcription 3;
Suppressor of cytokine signaling 3;
Prognosis

**Summary**
*Background and aims:* Hepatocellular carcinoma (HCC) is a complex and heterogeneous tumor with several genomic alterations, while the viral-chemical etiology along with molecular mechanisms of HCC pathogenesis remains largely unknown. This study aimed to determine expression profile and prognostic value of HER-2/neu, STAT3 and SOCS3 in HCC.
*Methods:* Immunohistochemistry and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) were performed to evaluate the expression of HER-2/neu, STAT3 and SOCS3 in HCC tissues and adjacent normal tissues collected from 176 HCC patients.
*Results:* HER-2/neu and STAT3 levels were higher and SOCS3 expression was lower in HCC tissues than in adjacent normal tissues. HER-2/neu, STAT3 and SOCS3 levels were associated with histological grade, tumor diameter, TNM stage, vascular invasion, lymph node metastasis and distant metastasis in HCC. SOCS3 expression was negatively associated with HER-2/neu and STAT3 expression. HCC patients with higher HER-2/neu and STAT3 levels had shorter overall, disease-free and disease-specific survival, whereas the opposite was found in patients with higher SOCS3 expression. In Cox regression analysis, tumor size, TNM stage, and STAT3 expression were identified as independent prognostic factors of HCC.
*Conclusion:* Taken together, these observations suggest that HER-2/neu, STAT3 and, SOCS3 are related to the aggressive tumor behavior and STAT3 has potential value as a prognostic factor for HCC.


## Introduction

Hepatocellular carcinoma (HCC) is a malignant tumor type that occurs in patients with chronic liver disease and liver cirrhosis. HCC exhibits grave mortality and is the main

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cause of cancer death worldwide [1]. HCC patients present multiple symptoms, such as obstructive jaundice, ascites, fatigue, weight loss, depression and extreme pain [2—5]. The occurrence and development of HCC can be activated by various factors, including excessive alcohol consumption, hepatitis B and C viruses, genetic disorders and obesity/diabetes [6]. The currently available therapeutics for HCC are radiotherapy, chemotherapy, liver transplantation and hepatic resection [7], the latter of which remains the only option for early-stage HCC patients [8]. Due to its high recurrence rate, lack of effective therapies and difficulty of diagnosis, HCC is associated with a very poor patient prognosis (the 5-year survival rate is only 7%) [9]. Targeted molecular therapy has increasingly attracted attention in the field of carcinoma biology as a way to improve the efficacy and reduce the toxicity of anti-cancer treatment [10]. Thus, further explorations of the molecular alterations in HCC are needed to identify new targets to improve diagnostic accuracy and treatment outcomes [4,13,17,20,30].

Human epidermal growth factor receptor-2 (HER-2/neu), a member of the human HER/EGFR/ERBB family, aggravates normal cellular function and organismal development [11]. HER-2/neu was reported to be an important biomarker and therapeutic target in about 30% of breast cancer patients [12—14]. In addition, the prognosis was found to be generally poor for patients with abnormal HER-2/neu expression in HCC tissues [15]. Signal transducer and activator of transcription 3 (STAT3), a member of the STAT family that promotes cytokine production, was shown to intensify cellular proliferation and apoptosis in several cancer types [16—18]. Recent research has suggested that STAT3 is abnormally activated in solid malignancies, and that blocking aberrant STAT3 signaling could be a novel therapeutic strategy that suppresses HCC cell proliferation, angiogenesis, survival, metastasis and progression [19—21].

STAT3 activation is associated with the expression of suppressor of cytokine signaling 3 (SOCS3), a negative feedback protein that induces STAT3 downstream of the gp130 signal [22]. The loss of SOCS3 expression was found to be associated with basal breast carcinoma, and SOCS3 methylation was shown to be associated with poor prognoses and clinical outcomes in various hepatic cancers [23]. Based on the above, the expression of HER-2/neu, STAT3 and SOCS3 could be involved in HCC. Therefore, we explored the relationship between HER-2 and the STAT3/SOCS3 signaling pathway, and the association of these proteins with the clinicopathological features and prognoses of HCC patients.

## Materials and methods

### Ethical statement

This study was performed according to the guidelines of the Ethics Committee of the Affiliated Yantai Yuhuangding Hospital of Qingdao University, and all the patients understood and agreed to participate in the study.

### Study subjects

From June 2007 to November 2009, 176 patients (males: $n = 148$; females, $n = 28$; age range: 25—78 years; mean age: $51.4 \pm 8.3$ years) pathologically diagnosed with HCC [24] at the Affiliated Yantai Yuhuangding Hospital of Qingdao University were included for the collection of the 176 samples in this study. The inclusion criteria were:

- HCC tissue sections collected from patients with complete follow-up data;
- samples collected after the patients had undergone radical resection and lymph node dissection;
- samples collected from patients who had not received any radiotherapy or chemotherapy.

Of the 176 HCC patients, 47 were infected with Hepatitis B virus (HBV), though the HBV was independent of HCC development. Among the 176 HCC samples, based on Edmondson—Steiner grading [25], 114 were well differentiated (I—II) and 62 were poorly differentiated (III—IV). According to the TNM staging system [26], 92 cases were at stages I-II and 84 cases were at stages III-IV. The histopathological features (tumor size, lymph node metastasis, vascular invasion, distant metastasis, etc.) are shown in Table 1. Adjacent normal tissues were collected from all 176 HCC patients. The fresh tissues were fixed in 10% formalin, embedded in paraffin and sectioned. Immunohistochemical staining was then carried out [2,3,12,16,19,29].

### Immunohistochemistry

All the slides were deparaffinized in a 65 °C oven for 2 h, dewaxed three times with xylene (15 min each time) and subjected to gradient ethanol dehydration. The slides were rinsed with distilled water, and peroxidase was removed with 3% hydrogen peroxide. All the slides were kept in citrate buffer at a high temperature and high pressure for antigen retrieval for 2 min, rinsed with distilled water to cool to room temperature, and allowed to stand for 30 min. After 3-min rinsing of the slides with phosphate-buffered saline (PBS) three times, mouse primary antibodies to HER-2/neu, STAT3 and SOCS3 were added (1 : 1000; Abcam Inc., Cambridge, MA, UK) and the slides were incubated at 37 °C for 1 h. After three 3-min rinses with PBS, the secondary horseradish peroxidase-labeled rabbit/mouse antibodies were added (Dako REAL™, Santa Clara, CA, USA; EnVison™). The slides were then washed three times with PBS (3 min each time), developed with 3,3′-diaminobenzidine for 5—10 min at room temperature, and washed under running water. The slides were counterstained with hematoxylin, dehydrated with ethanol, cleared with xylene, and sealed and fixed with resin. Samples that were not treated with mouse anti-HER-2/neu, STAT3 and SOCS3 were used as negative controls.

The results were judged by the following criteria. Pale yellow, brown and deeper brown particles in the cytoplasm or cytomembrane were considered to indicate positive expression. HER-2/neu positivity was judged based on cytomembrane expression. SOCS3 and STAT3 positivity were determined from cytoplasmic expression, and pale yellow, brown and deeper brown particles were regarded as the positive cells. Cells without staining or with less than 10% staining in the cytoplasm (−) and cells with greater than 10% incomplete staining in the cytoplasm (+) were considered to

**Table 1** Relationship between the expression of HER-2/neu and clinicopathological features of HCC patients.

| Clinical parameter | Sample number (%) | HER-2/neu | | *P* |
|---|---|---|---|---|
| | | Positive ($n = 83$) | Negative ($n = 93$) | |
| Age (year) | | | | 0.502 |
| ≤ 55 | 121 (68.8%) | 55 (45.5%) | 66 (54.5%) | |
| > 55 | 55 (31.3%) | 28 (50.9%) | 27 (49.1%) | |
| Gender | | | | 0.933 |
| Male | 148 (84.1%) | 70 (47.3%) | 78 (52.7%) | |
| Female | 28 (15.9%) | 13 (46.4%) | 15 (53.6%) | |
| Degree of differentiation | | | | < 0.001[a] |
| Well differentiated | 114 (64.8%) | 40 (33.6%) | 74 (62.2%) | |
| Poor differentiated | 62 (35.2%) | 43 (75.4%) | 19 (33.3%) | |
| Tumor diameter (cm) | | | | < 0.001[a] |
| > 5 | 136 (77.3%) | 74 (53.6%) | 62 (44.9%) | |
| ≤ 5 | 40 (22.7%) | 9 (23.7%) | 31 (81.6%) | |
| Lymph node metastasis | | | | < 0.001[a] |
| Yes | 96 (54.5%) | 65 (64.4%) | 31 (30.7%) | |
| No | 80 (45.5%) | 18 (24.0%) | 62 (82.7%) | |
| TNM staging | | | | 0.001[a] |
| I/II | 92 (52.3%) | 32 (36.0%) | 60 (67.4%) | |
| III/IV | 84 (47.7%) | 51 (58.6%) | 33 (37.9%) | |
| Vascular invasion | | | | < 0.001[a] |
| Yes | 117 (66.5%) | 66 (56.4%) | 51 (43.6%) | |
| No | 59 (33.5%) | 17 (28.8%) | 42 (71.2%) | |
| Distant metastasis | | | | < 0.001[a] |
| Yes | 83 (47.2%) | 57 (68.7%) | 26 (31.3%) | |
| No | 93 (52.8%) | 26 (28.0%) | 67 (72.0%) | |
| HBV infection | | | | 0.794 |
| Yes | 129 (73.3%) | 66 (51.2%) | 63 (48.8%) | |
| No | 47 (26.7%) | 17 (36.2%) | 30 (63.8%) | |

HER-2/neu: human epidermal growth factor receptor-2; HCC: hepatocellular carcinoma; TNM: tumor node metastasis.
[a] *P*-values after Bonferroni correction.

have negative expression, while those with greater than 10% weak but complete staining in the cytoplasm (++) or greater than 10% strong and complete staining in the cytoplasm (+++) were considered to have positive expression.

## Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from 0.1 g of HCC tissue or adjacent normal tissue with TRIZOL reagent (Invitrogen Inc., Carlsbad, CA, USA) and reverse- transcribed into cDNA with a First Strand cDNA Synthesis Kit (Takara Biotechnology Ltd., Dalian, Liaoning, China). RT-qPCR was carried out to determine mRNA levels. The primer sequences for HER-2/neu, STAT3 and SOCS3 were as follows: for HER-2/neu, forward, 5′-CCTCTGACGTCCATCGTCTC-3′ and reverse, 5′-CGGATCTTCTGCTGCCGTCG-3′; for *STAT3*, forward, 5′-TAGCAGGATGGCCCAATGGAATCA-3′ and reverse, 5′-AGCTGTCACTGTAGAGCTGATGGA-3′; for *SOCS3*, forward, 5′-TCCCCCCAGAAGAGCCTATTAC-3′ and reverse, 5′-TCCGACAGAGATGCTGAAGAGTG-3′. The primer sequences for the internal control (β-actin) were as follows: forward, 5′-GCTATCCAGGCTGTGCTATC-3′ and reverse, 5′-ACTGTGTTGGCGTACAGGTC-3′. RT-qPCR was performed with an ABI 7500 PCR machine (ABI Company, Oyster Bay, N.Y., USA). The mixture was purchased from Bio-Rad (Bio-Rad, Inc., Hercules, CA, USA). The PCR reaction conditions were as follows: pre-denaturation at 94 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 1 min. The formula for determining the differential expression of mRNA was as follows: $N = (1 + E)^{-\Delta\Delta CT}$, where E (defined as the amplification efficiency of the primers) was equal to 1 in this study.

## Follow-up

The follow-up study was conducted from January 1, 2011 to January 1, 2018, and the median follow-up time was 56.5 months (10—84 months). Patients with signs of new recurrence or other complications were recorded. The overall survival (OS), disease-free survival (DFS) and disease specific survival (DSS) of patients with HCC were collected. OS was defined as the survival time between the start of HCC treatment and death from any cause. DFS was defined as the survival time between the start of HCC treatment and the occurrence of other complications. DSS was defined as

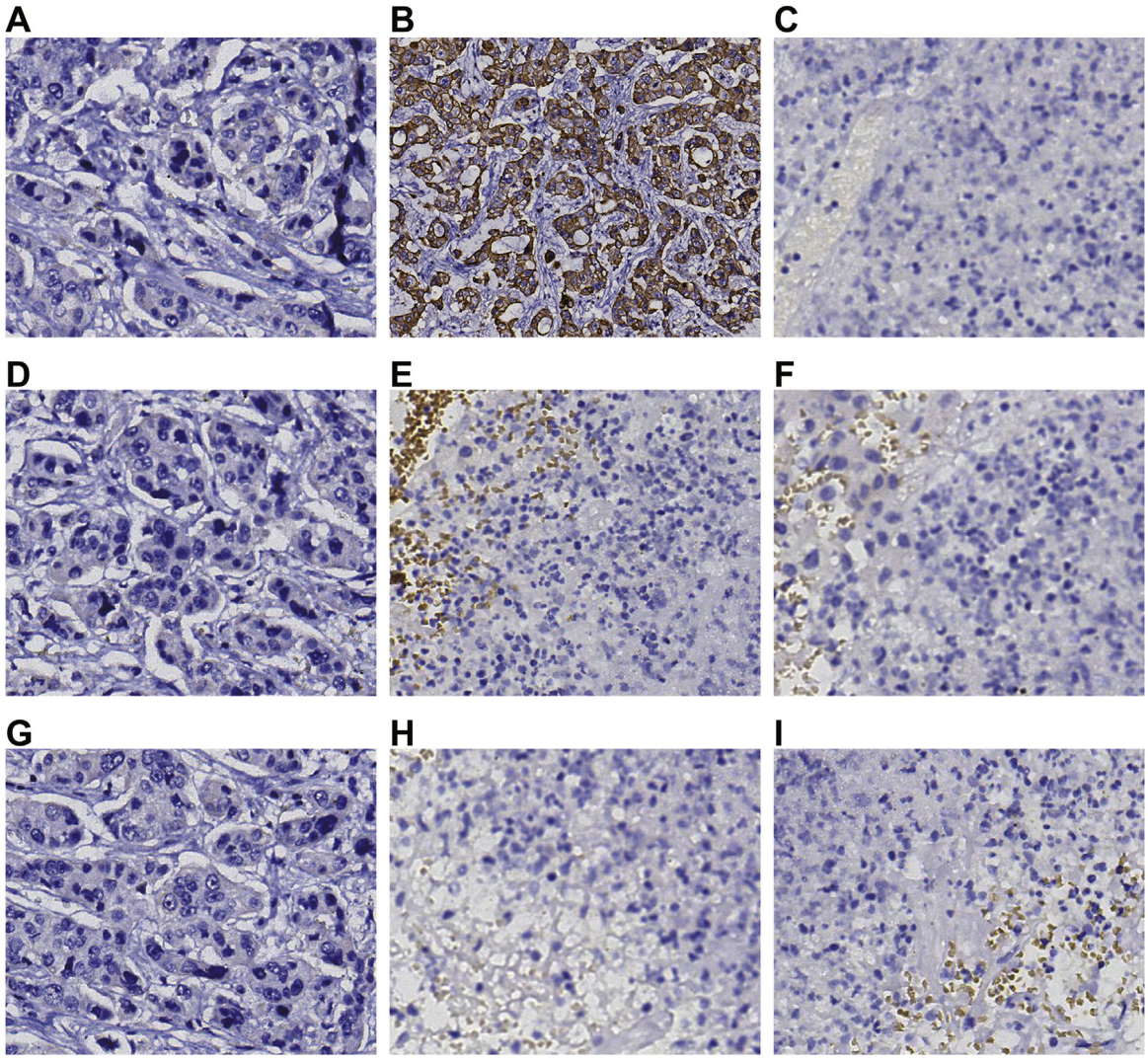


**Figure 1** HER-2/neu and STAT3 is overexpressed and SOCS3 is downregulated in HCC tissues (× 200). A. Negative control of HER-2/neu. B. HER-2/neu expression in HCC tissue (+++). C. HER-2/neu expression in adjacent normal tissue (−). D. Negative control of STAT3. E. STAT3 expression in HCC tissue (+++). F. STAT3 expression in adjacent normal tissue (−). G. Negative control of SOCS3. H. SOCS3 expression in HCC tissue (−). I. SOCS3 expression in adjacent normal tissue (+++); HCC: hepatocellular carcinoma; HER-2: human epidermal growth factor receptor-2; STAT3, signal transducer and activator of transcription 3; SOCS3, suppressor of cytokine signaling 3.

the survival time between the start of HCC treatment and death from HCC. During this follow-up, 140 patients died (79.5%). Among these 140 deaths, 4 (2.3%) were due to cardiovascular diseases or accidents. Thirty-six patients (20.5%) survived until the end of follow-up. Patents who were lost to follow-up, survived until the end of follow-up or died for other causes were considered as censored data.

### Statistical analysis

Data analysis was performed with SPSS 21.0 (IBM Corp. Armonk, NY, USA). The $Chi^2$ test was used to compare the expression of HER-2/neu, STAT3 and SOCS3 between HCC tissues and adjacent normal tissues, and to determine the correlation of their expression with HCC clinical features. Spearman correlation analysis was performed to assess the correlation between the levels of different proteins. The survival time was plotted as a Kaplan—Meier curve. Multivariate analysis of prognosis was performed with Cox's proportional hazards regression model and tested with the Wald test. $P < 0.05$ indicated a significant difference.

## Results

### Overexpressed HER-2/neu and STAT3 but downregulated SOCS3 is found in HCC tissues

Among 176 HCC tissues, 93 were HER-2/neu-negative and 83 were HER-2/neu-positive. No HER-2/neu-positive adjacent normal tissue was found. STAT3 protein expression was positive in 103 and negative in 73 HCC tissues, while it was positive in 48 and negative in 128 adjacent normal tissues.

**Table 2** Relationship between the expression of STAT3 and clinicopathological features of HCC patients.

| Clinical parameter | Sample number (%) | STAT3 | | *P* |
|---|---|---|---|---|
| | | Positive($n=103$) | Negative($n=73$) | |
| Age (year) | | | | 0.353 |
| ≤ 55 | 121 (68.8%) | 68 (56.2%) | 53 (43.8%) | |
| > 55 | 55 (31.3%) | 35 (63.6%) | 20 (36.4%) | |
| Gender | | | | 0.562 |
| Male | 148 (84.1%) | 88 (59.5%) | 60 (40.5%) | |
| Female | 28 (15.9%) | 15 (53.6%) | 13 (46.4%) | |
| Degree of differentiation | | | | 0.002 |
| Moderately differentiation | 114 (64.8%) | 57 (50.0%) | 57 (50.0%) | |
| Poorly differentiation | 62 (35.2%) | 46 (74.2%) | 16 (25.8%) | |
| Tumor diameter (cm) | | | | 0.002 |
| > 5 | 136 (77.3%) | 88 (64.7%) | 48 (35.3%) | |
| ≤ 5 | 40 (22.7%) | 15 (37.5%) | 25 (62.5%) | |
| Lymph node metastasis | | | | < 0.001[a] |
| Yes | 96 (54.5%) | 69 (71.9%) | 27 (28.1%) | |
| No | 80 (45.5%) | 34 (42.5%) | 46 (57.5%) | |
| TNM staging | | | | 0.003 |
| I/II | 92 (52.3%) | 44 (47.8%) | 48 (52.5%) | |
| III/IV | 84 (47.7%) | 59 (70.2%) | 25 (29.8%) | |
| Vascular invasion | | | | 0.015 |
| Yes | 117 (66.5%) | 76 (65.0%) | 41 (35.0%) | |
| No | 59 (33.5%) | 27 (45.8%) | 32 (54.2%) | |
| Distant metastasis | | | | < 0.001[a] |
| Yes | 83 (47.2%) | 62 (74.7%) | 21 (25.3%) | |
| No | 93 (52.8%) | 41 (44.1%) | 52 (55.9%) | |
| HBV infection | | | | 0.864 |
| Yes | 129 (73.3%) | 75 (58.1%) | 54 (41.9%) | |
| No | 47 (26.7%) | 28 (59.6%) | 19 (40.4%) | |

STAT3: signal transducer and activator of transcription 3; HCC: hepatocellular carcinoma; TNM: tumor node metastasis.
[a] *P*-values after Bonferroni correction.

SOCS3 protein expression was positive in 93 and negative in 83 HCC tissues, while it was positive in 147 and negative in 29 adjacent normal tissues (Fig. 1). The expression of HER-2/neu, STAT3 and SOCS3 differed significantly between HCC tissues and adjacent normal tissues (all $P < 0.001$). In the RT-qPCR assay, the mRNA levels of HER-2/neu and STAT3 were higher in HCC tissues than in adjacent normal tissues, while the mRNA level of SOCS3 was significantly lower in HCC tissues than that in adjacent normal tissues (all $P < 0.001$) (Fig. 2). These findings indicated that HCC tissues exhibited overexpressed HER-2/neu and STAT3 but diminished SOCS3.

## The correlation between the expression of HER-2/neu, STAT3 and SOCS3 and clinicopathological features of HCC patients

No significant association of HER-2/neu, STAT3 or SOCS3 expression with age, gender or HBV was found (all $P > 0.05$). After Bonferroni correction, the expression of HER-2/neu in HCC patients was significantly associated with the degree of differentiation, tumor diameter, tumor-node-metastasis (TNM) stage, vascular invasion, lymph node metastasis and distant metastasis (all $P < 0.05$) (Table 1); the expression of STAT3 in HCC patients was associated with lymph node metastasis and distant metastasis (both $P < 0.05$) (Table 2); and the expression of SOCS3 in HCC patients was related to the degree of differentiation, vascular invasion, lymph node metastasis and distant metastasis (all $P < 0.05$) (Table 3).

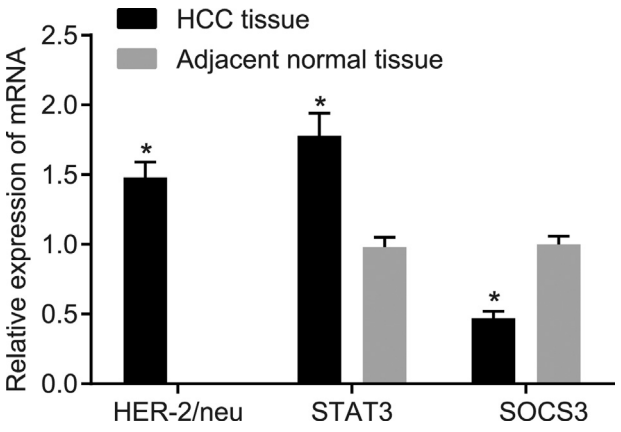


**Figure 2** The mRNA levels of HER-2/neu, STAT3 and SOCS3 between HCC tissues ($n = 176$) and the corresponding adjacent normal tissues ($n = 176$), detected by RT-qPCR. The mRNA levels of STAT3 and SOCS3 in adjacent normal tissues were set to 1; *, $P < 0.01$ vs. adjacent normal tissues.

**Table 3** Relationship between the expression of SOCS3 and clinicopathological features of HCC patients.

| Clinical parameter | Sample number (%) | SOCS3 | | *P* |
|---|---|---|---|---|
| | | Positive ($n=82$) | Negative ($n=94$) | |
| Age (years) | | | | 0.984 |
| ≤ 55 | 121 (68.8%) | 64 (52.89%) | 57 (47.11%) | |
| > 55 | 55 (31.3%) | 29 (52.73%) | 26 (47.27%) | |
| Gender | | | | 0.933 |
| Male | 148 (84.1%) | 78 (52.70%) | 70 (47.30%) | |
| Female | 28 (15.9%) | 15 (53.57%) | 13 (46.43%) | |
| Degree of differentiation | | | | 0.001[a] |
| Moderately differentiation | 114 (64.8%) | 71 (62.28%) | 43 (37.72%) | |
| Poorly differentiation | 62 (35.2%) | 22 (35.48%) | 40 (64.52%) | |
| Tumor diameter (cm) | | | | 0.013 |
| > 5 | 136 (77.3%) | 65 (47.79%) | 71 (52.21%) | |
| ≤ 5 | 40 (22.7%) | 28 (70.00%) | 12 (30.00%) | |
| Lymph node metastasis | | | | < 0.001[a] |
| Yes | 96 (54.5%) | 32 (33.33%) | 64 (66.67%) | |
| No | 80 (45.5%) | 61 (76.25%) | 19 (23.75%) | |
| TNM staging | | | | 0.005 |
| I/II | 92 (52.3%) | 58 (63.04%) | 34 (36.96%) | |
| III/IV | 84 (47.7%) | 35 (41.67%) | 49 (58.33%) | |
| Vascular invasion | | | | < 0.001[a] |
| Yes | 117 (66.5%) | 50 (42.74%) | 67 (57.26%) | |
| No | 59 (33.5%) | 43 (72.88%) | 16 (27.12%) | |
| Distant metastasis | | | | < 0.001[a] |
| Yes | 83 (47.2%) | 28 (33.3%) | 55 (66.27%) | |
| No | 93 (52.8%) | 65 (69.89%) | 28 (30.11%) | |
| HBV infection | | | | 0.460 |
| Yes | 129 (73.3%) | 66 (51.2%) | 63 (48.8%) | |
| No | 47 (26.7%) | 27 (57.5%) | 20 (42.5%) | |

SOCS3: suppressor of cytokine signaling 3; HCC: hepatocellular carcinoma; TNM: tumor node metastasis.
[a] *P*-values after Bonferroni correction.

## STAT3 is positively correlated while SOCS3 is negatively correlated to HER-2/neu

In Spearman correlation analysis, 68 STAT3-positive samples and 19 SOCS3-positive sample were identified among the 83 HER-2/neu-positive HCC tissues (Table 4). STAT3 expression correlated positively with HER-2/neu expression in HCC tissues ($P < 0.001$), while SOCS3 expression correlated negatively with HER-2/neu expression in HCC tissues ($P < 0.001$).

## HER-2/neu, STAT3 and SOCS3 expression are associated with HCC patient survival

Follow-up was performed for 176 HCC patients. Kaplan-Meier curves revealed that the OS, DFS and DSS were significantly shorter in HER-2/neu-positive patients than in HER-2/neu-negative patients (OS: 45 months vs. 73 months; DFS: 38 months vs. 60 months; DSS: 45 months vs. 74 months) ($P < 0.001$) (Fig. 3A, D and G). Likewise, the median OS, DFS and DSS were shorter in STAT3-positive patients than in STAT3-negative patients (OS: 46 months vs. 76 months; DFS: 40 months vs. 65 months; DSS: 46 months vs. 76 months) (Fig. 3B, E and H). However, the median OS, DFS and DSS were longer in SOCS3-positive patients than in SOCS3-negative patients (OS: 68 months vs. 42 months; DFS: 60 months vs. 37 months; DSS: 69 months vs. 42 months) (Fig. 3C, F and I). Thus, the Kaplan—Meier survival analysis indicated that the survival times were much shorter for HER-2/neu-positive, STAT3-positive and SOCS3-negative HCC patients than for HER-2/neu-negative, STAT3-negative and SOCS3-positive HCC patients (all $P < 0.001$).

In Cox regression analysis, the tumor size, number of lymph node metastasis, TNM staging and protein expression of HER-2/neu, STAT3 and SOCS3 were identified as potential independent prognostic factors for the OS of HCC patients (all $P < 0.05$), but vascular invasion and the Edmondson—Steiner grade were not (both $P > 0.05$). The tumor size, TNM staging and protein expression of HER-2/neu and STAT3 were identified as potential independent prognostic factors for the DFS of HCC patients (all $P < 0.05$), whereas SOCS3 protein expression, the number of lymph node metastasis, vascular invasion and the Edmondson—Steiner grade were not (all $P > 0.05$). The tumor size, number of lymph node metastasis, TNM staging and protein expression of STAT3 and SOCS3 were identified as potential independent prognostic factors for the DSS of HCC patients (all $P < 0.05$), while HER-2/neu protein expression, vascular invasion and the Edmondson—Steiner grade were not (all $P > 0.05$) (Table 5).

**Table 4** Correlation among the expression of HER-2/neu, STAT3 and SOCS3 in HCC tissues.

| HER-2/neu | STAT3 | | | | SOCS3 | | | |
|---|---|---|---|---|---|---|---|---|
| | — | + | ++ | +++ | — | + | ++ | +++ |
| — | 14 | 19 | 14 | 4 | 2 | 8 | 25 | 16 |
| + | 7 | 18 | 9 | 8 | 5 | 4 | 18 | 15 |
| ++ | 5 | 6 | 33 | 21 | 18 | 33 | 12 | 2 |
| +++ | 1 | 3 | 9 | 5 | 4 | 9 | 4 | 1 |

HCC: hepatocellular carcinoma; HER-2/neu: human epidermal growth factor receptor-2; STAT3: signal transducer and activator of transcription 3; SOCS3: suppressor of cytokine signaling 3.

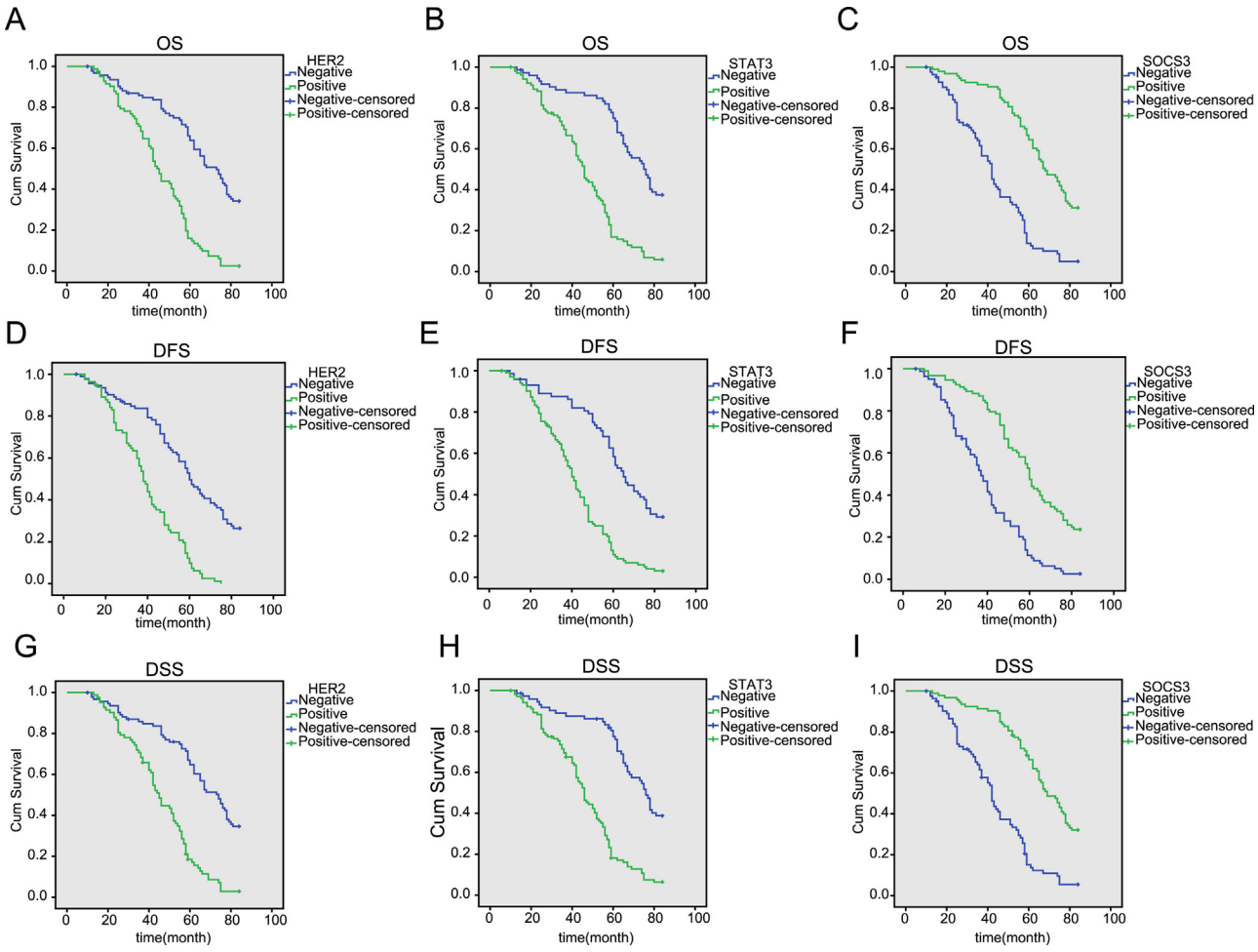


**Figure 3** Kaplan—Meier curves for the expression of HER-2/neu, STAT3 and SOCS3 in 176 HCC patients. A. OS curves of HER-2/neu-positive/negative HCC patients. B. OS curves of STAT3-positive/negative HCC patients. C. OS curves of SOCS3- positive/negative HCC patients. D. DFS curves of HER-2/neu-positive/negative HCC patients. E. DFS curves of STAT3-positive/negative HCC patients. F. DFS curves of SOCS3-positive/negative HCC patients. G. DSS curves of HER-2/neu-positive/negative HCC patients. H. DSS curves of STAT3-positive/negative HCC patients. I. DSS curves of SOCS3-positive/negative HCC patients. HCC: hepatocellular carcinoma; HER-2: human epidermal growth factor receptor-2; STAT3: signal transducer and activator of transcription 3; SOCS3: suppressor of cytokine signaling 3; OS: overall survival; DFS: disease-free survival; DSS: disease-specific survival.

## Discussion

HCC is one of the most common malignancies around the world, with about one million new cases per year [27]. For years, the most efficient therapies for HCC have been surgical resection and liver transplantation, but the recurrence rates of HCC have remained high [28]. Decades of extensive studies have demonstrated that many molecules relevant to cell migration contribute to tumor invasion and poor prognoses in HCC [29—31]. Abnormal HER-2/neu, STAT3 and SOCS3 expression could promote cell growth, inhibit apop-

**Table 5** Cox regression analysis of independent prognostic factors for 176 HCC patients.

| Independent prognostic factors | OR | 95% CI | *P* |
|---|---|---|---|
| OS | | | |
| HER-2/neu | 1.683 | 1.051—2.695 | 0.030 |
| SOCS3 | 0.619 | 0.387—0.987 | 0.044 |
| STAT3 | 1.762 | 1.101—2.820 | 0.018 |
| Tumor size | 1.690 | 1.095—2.608 | 0.018 |
| Number of lymph node metastasis | 1.643 | 1.087—2.483 | 0.019 |
| Vascular invasion | 0.942 | 0.627—1.416 | 0.775 |
| TNM staging (III/IV) | 1.697 | 1.136—2.536 | 0.010 |
| Edmondson—Steiner grade | 1.181 | 0.769—1.812 | 0.448 |
| DFS | | | |
| HER-2/neu | 1.697 | 1.056—2.728 | 0.029 |
| SOCS3 | 0.776 | 0.486—1.239 | 0.288 |
| STAT3 | 1.824 | 1.139—2.920 | 0.012 |
| Tumor size | 1.635 | 1.067—2.507 | 0.024 |
| Number of lymph node metastasis | 1.324 | 0.882—1.987 | 0.176 |
| Vascular invasion | 0.969 | 0.654—1.434 | 0.873 |
| TNM staging (III/IV) | 1.740 | 1.194—2.535 | 0.004 |
| Edmondson—Steiner grade | 1.017 | 0.674—1.536 | 0.935 |
| DSS | | | |
| HER-2/neu | 1.615 | 1.001—2.605 | 0.050 |
| SOCS3 | 0.620 | 0.387—0.994 | 0.047 |
| STAT3 | 1.832 | 1.137—2.952 | 0.013 |
| Tumor size | 1.632 | 1.054—2.526 | 0.028 |
| Number of lymph node metastasis | 1.672 | 1.097—2.546 | 0.017 |
| Vascular invasion | 1.010 | 0.666—1.532 | 0.962 |
| TNM staging (III/IV) | 1.168 | 2.632 | 0.007 |
| Edmondson—Steiner grade | 1.236 | 0.799—1.912 | 0.342 |

HCC: hepatocellular carcinoma; HER-2: human epidermal growth factor receptor-2; STAT3: signal transducer and activator of transcription 3; SOCS3: suppressor of cytokine signaling 3; OR: odds ratio; CI: confidence interval; TNM: tumor node metastasis; OS: overall survival; DFS: disease-free survival; DSS: disease-specific survival; OR: odds ratio (for a disease with a very low incidence, it is a OR value that is an accurate estimate of the relative risk).

tosis and promote cellular invasion and metastasis in HCC [32,33]. In this study, we investigated the effects of HER-2/neu and JAK/STAT3-SOCS3 signaling on HCC by exploring the levels of these proteins in HCC tissues and their association with the clinic pathological features and prognoses of HCC patients.

Our study revealed that HER-2/neu and STAT3 protein levels were significantly higher in HCC tissues than in adjacent normal tissues, while SOCS3 protein levels were lower in HCC tissues. Furthermore, in HCC patients, the overexpression of HER-2/neu and STAT3 were associated with lower OS, while SOCS3 positivity was associated with longer survival than SOCS3 negativity. Cox regression analysis also indicated that the tumor size, TNM stage, number of lymph node metastases and expression of HER-2/neu, STAT3 and SOCS3 were potential independent prognostic factors predicting the OS of HCC patients. Thus, HER-2/neu, STAT3 and SOCS3 expression may be significant features for the prognosis and clinical assessment of HCC, and could be novel molecular targets for HCC treatment.

Consistent with these results, a previous study revealed that HER-2/neu overexpression increased the invasive potential of breast and non-small cell lung cancer cells, indicating that HER-2/neu could be a candidate gene for targeted therapy in HCC [34]. Another study demonstrated that HER-2/neu overexpression was an indicator of poor prognosis in HBx-expressing HCC patients [35]. The cooperation between EGFR and HER-2/neu was reported to promote cancer cell progression and tumor growth, and targeted therapy was recommended for patients with HER-2 protein overexpression and gene amplification [36].

STAT3 is a nuclear transcription factor, which is the main component of the JAK/STAT3 signaling transduction pathway [37]. After being stimulated by extracellular signals such as growth factors and cytokines, it enters the nucleus and binds to its specific DNA sequence to regulate the transcription of target genes, thus affecting cell proliferation, differentiation and apoptosis [38]. In recent years, STAT3 has been regarded as an oncogene and abnormal expression of STAT3 has been found to be related to the occurrence, development and metastasis of tumor in many malignant tumors [39]. Additionally, the overexpression of STAT3 was shown to promote the maintenance and proliferation of human cancer-initiating cells, thus increasing the incidence rates of various malignant cancers [40]. Dysregulated STAT3 signaling was reported to promote tumorigenesis by stimulating cancer cell activities, and transient STAT3 activation was reported to cause cancer-cell resistance to drug treatment [41]. Sum et al. demonstrated that STAT3 signaling was associated with EGFR (a proto-oncogene) and thus pro-

moted cellular proliferation, angiogenesis and metastasis [42]. These results agree with the findings of the present study.

Low expression of SOCS3 may also promote malignancies and carcinogenesis by inducing multiple tumor-promoting genes [43]. SOCS3 was shown to negatively regulate STAT3 by knocking down its recruitment sites in HCC, while activated STAT was shown to suppress immune responses in different types of cancer [43]. Let-7 re-expression was reported to upregulate SOCS3, which inhibited cancer cell differentiation and proliferation [44]. In addition, high SOCS3 expression was shown to be associated with reduced tumor growth and metastasis [45]. Consistent with our study, the study reported by Nakagawa et al. revealed that reduced SOCS3 expression was the best indicator of lymph mode metastasis in breast cancer patients [46]. Therefore, SOCS3 can be regarded as an important index of HCC prognosis.

## Conclusion

Taken together, our results demonstrated that HER-2/neu, SOCS3 and STAT3 expression were associated with the progression of HCC. Overexpression of HER-2 and STAT3 and low expression of SOCS3 resulted in a poor prognosis in HCC patients. Therefore, HER-2, STAT3 and SOCS3 could be crucial prognostic detectors and molecular markers for the effective treatment of HCC.

## Disclosure of interest

The authors have declared that no competing interests exist.

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