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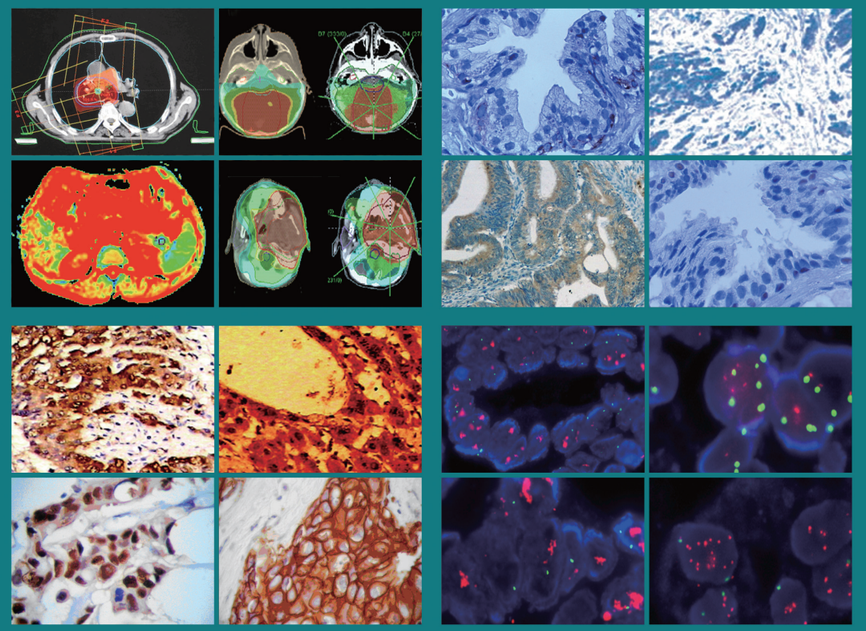

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Aims & Scope

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ORIGINAL ARTICLE

Survival outcomes of patients with cervical esophageal cancer who received definitive radiotherapy: a retrospective study conducted in a single institution*

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Abstract

Objective Cervical esophageal cancer (CEC) is a relatively rare condition, with limited treatment options. The current study aimed to assess the survival outcomes of patients with CEC who received definitive radiotherapy.

Methods In total, 63 consecutive patients with CEC who received definitive radiotherapy between 2010 and 2018 were included in this study. The survival outcomes were analyzed based on statistics.

Results The median progression-free survival (PFS) and overall survival (OS) of the patients were 12 and 19 months, respectively. There were no significant differences in terms of survival outcomes between the groups who received radiation doses ≥ 60 and < 60 Gy. Interestingly, in the proximal CEC subgroup, the PFS (P = 0.039), OS (P = 0.031), and loco-regional failure-free survival (LRFFS) (P = 0.005) improved significantly in patients who received a radiation dose ≥ 60 Gy compared with those who received a radiation dose < 60 Gy. However, in the distal CEC subgroup, the PFS, OS, and LRFFS did not significantly improve between patients who received radiation doses ≥ 60 and < 60 Gy. Definitive radiotherapy was well tolerated, and no significant differences were observed in terms of treatment-related toxicities between the groups who received radiation doses ≥ 60 and < 60 Gy.

Conclusion The survival outcomes of patients with CEC should be improved. In proximal CEC, a radiation dose ≥ 60 Gy is significantly correlated with better PFS, OS, and LRFFS. However, further research must be performed to validate this finding.

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Key words: cervical esophageal cancer; definitive radiotherapy; survival outcomes

Cervical esophageal cancer (CEC) is a relatively rare condition, which accounts for 2%–10% of all esophageal carcinomas [1]. Moreover, it is highly prevalent in Eastern Asia and Southern Africa [2]. This condition is defined as a tumor of the esophagus located between the cricoid cartilage and the sternal notch [3]. CEC is commonly diagnosed at a locally advanced stage, and the prognosis is poor, with a 5-year overall survival (OS) rate of 30%–48.3% [4]. Recently, pharyngo-laryngo-esophagectomy is performed for the treatment of such condition. However, the procedure is extensive and it often causes severe complications. Organ-sparing definitive concurrent chemoradiotherapy (dCCRT) is the standard treatment for CEC. Further, it is recommended by the National Comprehensive Cancer Network (NCCN) and European Society for Medical Oncology (ESMO) guidelines [5–6]. Nonetheless, over the past several decades, the survival of patients with CEC has not significantly improved [7].

Due to the low incidence of CEC, studies about this condition are relatively limited. Thus, there is no consensus whether dCCRT can be considered an optimal treatment regimen for CEC. The treatment of CEC is often referred to the esophageal cancer of other sites or hypopharyngeal carcinoma. The randomized phase III INT-0123/RTOG 94-05 trial compared the efficacy of standard-dose radiation (50.4 Gy) versus high-dose radiation (64.8 Gy) for the

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treatment of esophageal cancer. Results showed that dose escalation could not improve local/regional control or survival [8]. However, more than 85% of the patients were diagnosed with adenocarcinoma at various sites of the esophagus. Hence, the results were not applicable to CEC considering that 95% of the cases involved squamous cell carcinoma (SCC). Since the definitive radiotherapy dose for hypopharyngeal carcinoma is up to 70 Gy, some researchers recommend that the standard dose for CEC should be > 50 Gy [9]. Moreover, several studies have reported that a higher local-regional control rate [10–11] and better OS were observed in CEC patients who received a radiation dose > 50.4 Gy [12–13]. However, some studies have contrasting results [14].

To date, an optimal treatment protocol with adequate survival and acceptable toxicity for patients with CEC has not yet been established. To shed light on this issue, the current retrospective study aimed to investigate the survival outcomes of CEC patients who received definitive radiotherapy with different radiation doses.

Patients and methods

Patients

Between January 2010 and March 2018, 63 consecutive CEC patients who received definitive radiotherapy at the Oncology Center of Tongji Hospital, Wuhan, were included in this study. The participants were pathologically diagnosed with SCC. Each patient underwent contrast-enhanced computed tomography (CT) scan or magnetic resonance imaging (MRI) of the cervical spine, chest, abdomen, and brain; bone scan; or ^{18}FDG-positron emission tomography (PET)-CT scan. Cancer staging was performed using the 7th edition of the American Joint Committee on Cancer staging system. The study protocol was approved by the ethics committee of Tongji Hospital.

Treatment details

The patients received intensity-modulated radiotherapy (IMRT) or three-dimensional conformal radiotherapy. Gross tumor volume (GTV) was defined as the volume of the primary tumor and involved lymph nodes based on imaging modalities at diagnosis, including neck/chest CT scan, barium swallow, laryngoscopy, endoscopy/endoscopic ultrasound, and PET-CT scan. The prescription dose for GTV ranged from 50 to 70 Gy for over 5–7 weeks in 25–35 fractions with 5 fractions per week. Clinical tumor volume (CTV) was defined as GTV plus a margin of 3–5 cm longitudinally and 0.7–1 cm radially. The supraclavicular node areas and upper mediastinal areas were also included in the CTV for involved field irradiation (IFI) or elective nodal irradiation (ENI), with a prescription dose of 45–54 Gy. For daily set-up errors in radiation, the planning target volume was defined as CTV plus a margin of 0.5–1.0 cm.

Most patients (n = 42, 66.7%) were treated with concurrent chemoradiotherapy (CCRT). The most common regimen was cisplatin/5-fluorouracil-based chemotherapy (29/42, 69.0%). Meanwhile, the other treatments included oral capecitabine or S1. However, only some patients received radiotherapy due to poor performance or intolerance to chemotherapy.

Follow-up

The median follow-up time was 16 (range: 3–42.0) months. During treatment, the patients were monitored at least once a week to assess for treatment-related toxicities, which were evaluated and scored according to the Common Terminology Criteria for Adverse Events version 4.0. Treatment response was assessed after definitive radiotherapy using imaging modalities, including contrast-enhanced CT scan of the neck and thorax, according to the Response Evaluation Criteria in Solid Tumors version 1.1. After treatment, all patients were followed-up every 3 months within the first 2 years and once every 6 months thereafter. During each follow-up, to evaluate for toxicities and treatment response, the patients underwent physical examination, blood test, and imaging, including CT scan, MRI, ultrasonography, and endoscopy with or without biopsy.

Treatment failure was defined as the persistence or recurrence of the primary lesions or appearance of a new lesion. The failure patterns were identified based on the sites of first failure. Local and regional failure was defined as failure of treatment for the primary tumor or regional lymph nodes. Distant failure was defined as metastasis beyond the primary tumor and regional lymph nodes.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences software version 18.0 (SPSS, Chicago, IL, the USA). The progression-free survival (PFS), loco-regional failure-free survival (LRFFS), distant metastasis-free survival (DMFS), and overall survival (OS) were assessed using the Kaplan–Meier method. Univariate analyses were conducted to identify potential prognostic factors, using $P < 0.10$ as the cutoff value for multivariate analyses. Subsequently, the Cox proportional hazard model was used in the multivariate analyses, and a P value < 0.05 was considered statistically significant. The characteristics and failure pattern were compared between the groups who received high- and standard-dose radiation using the Pearson's chi-square test.

Results

Characteristics of the participants

Most participants (n = 35, 55.5%) presented with stage III disease. Three patients with stage IV disease with bone metastasis at a single site were included, and these patients received definitive radiotherapy. In total, 12 (19.0%) patients presented with hoarseness at diagnosis. Of 63 patients, 56 (88.9%) received IMRT and 7 3DCRT. The median GTV radiation dose was 60 (range: 50–70) Gy in 25–35 fractions. Moreover, 34 (60.7%) and 22 (39.3%) patients received radiations dose ≥ 60 and < 60 Gy, respectively. Of 63 patients, 51 (81.0%) received ENI. The characteristics of the patients are presented in Table 1. No significant difference was observed in terms of characteristics between the groups who received GTV radiation doses ≥ 60 and < 60 Gy (P > 0.05).

Survival analysis and radiation dose

In total, 55 patients died from treatment failure and other non-tumor causes during the follow-up period. The median OS was 19 months; median PFS, 12 months; median LRFFS, 12 months; and median DMFS, 13 months. The survival curves are depicted in Fig. 1.

Based on the Kaplan–Meier analysis using the log-rank test, no significant differences were found in terms of PFS (P = 0.053), OS (P = 0.300), LRFFS (P = 0.193), and DMFS (P = 0.175) between the groups who received radiation doses ≥ 60 Gy and < 60 Gy.

Similar to the effect of radiation dose on PFS, we further divided the patients into the proximal and distal CEC subgroups according to the location of the tumor above or below the middle portion of the cervical esophagus. There were 36 patients in the proximal CEC subgroup and 27 in the distal CEC subgroup. In total, 24 (66.7%) patients in the proximal CEC subgroup and 12 (44.4%) in the distal CEC subgroup received an RT dose ≥ 60 Gy. In the proximal CEC subgroup, six (50%) patients with stage I-II disease and three (25%) without concurrent chemotherapy received an RT dose < 60 Gy. Meanwhile, 11 (45.8%) patients with stage I-II disease and 7 (29.2%) without concurrent chemotherapy received an RT dose ≥ 60 Gy. In the distal CEC subgroup, five (33.3%) patients with stage I-II disease and seven (46.7%) without concurrent chemotherapy received an RT dose < 60 Gy. Meanwhile, three (25.0%) patients with stage I-II and four (33.3%) without concurrent chemotherapy received an RT dose ≥ 60 Gy. Based on the Kaplan–Meier analysis, in the proximal CEC subgroup, a GTV radiation dose ≥ 60 Gy was significantly correlated with better PFS (P = 0.039), LRFFS (P = 0.005), and OS (P = 0.031), but not with DMFS (P= 0.107). The survival curves are presented in Fig. 2. However, in the distal CEV subgroup, the correlation was not significant (PFS, P = 0.131; LRFFS, P = 0.097; DMFS, P = 0.639; and OS, P = 0.132). The GTV and CTV for proximal and distal CEC are depicted in Fig. 3.

Table 1 Patients' characteristics

Characteristics	Radiation dose < 60 Gy (n = 27)	Radiation dose < 60 Gy (n = 36)	P value
Age (years)			0.710
≤ 60	17 (63.0%)	21 (58.3%)	
> 60	10 (37.0%)	15 (41.7%)	
Sex (n, %)			0.127
Male	19 (70.4%)	31 (86.1%)	
Female	8 (29.6%)	5 (13.9%)	
ECOG score			0.710
0–1	17 (63.0%)	21 (58.3%)	
2–3	10 (37.0%)	15 (41.7%)	
T classification (n, %)			0.798
T1–2	6 (22.2%)	9 (25.0%)	
T3–4	21 (77.8%)	27 (75.0%)	
N classification (n, %)			0.369
N0	12 (44.4%)	12 (33.3%)	
N+	15 (55.6%)	24 (66.7%)	
Tumor Location			0.078
Proximal	12 (44.4%)	24 (66.7%)	
Distal	15 (55.6%)	12 (33.3%)	

P < 0.05 was considered to indicate a statistically significant difference. n, number; ECOG, Eastern Cooperative Oncology Group

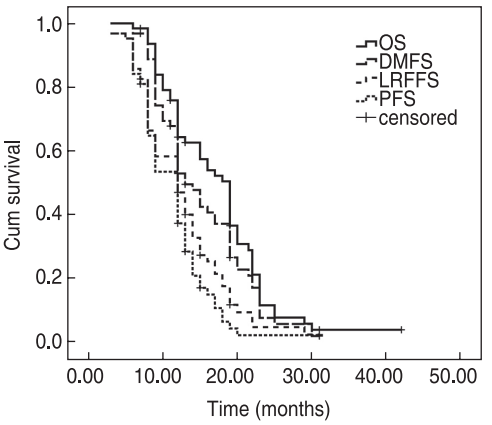

Fig. 1 Survival curves of progression-free survival, loco-regional failure-free survival, distant metastasis-free survival, and overall survival assessed using the Kaplan–Meier method in patients with cervical esophageal cancer

Prognostic factors

A univariate Cox analysis of clinical factors, including gender, age, fistula, neutrophil-to-lymphocyte ratio, hoarseness, T classification, N classification, TNM stage, and GTV radiation dose (cutoff of 60 Gy), was conducted. The results are presented in Table 2. Only hoarseness was significantly associated with worse PFS (P = 0.040), OS (P = 0.008), LRFFS (P = 0.035), and DMFS (P = 0.019).

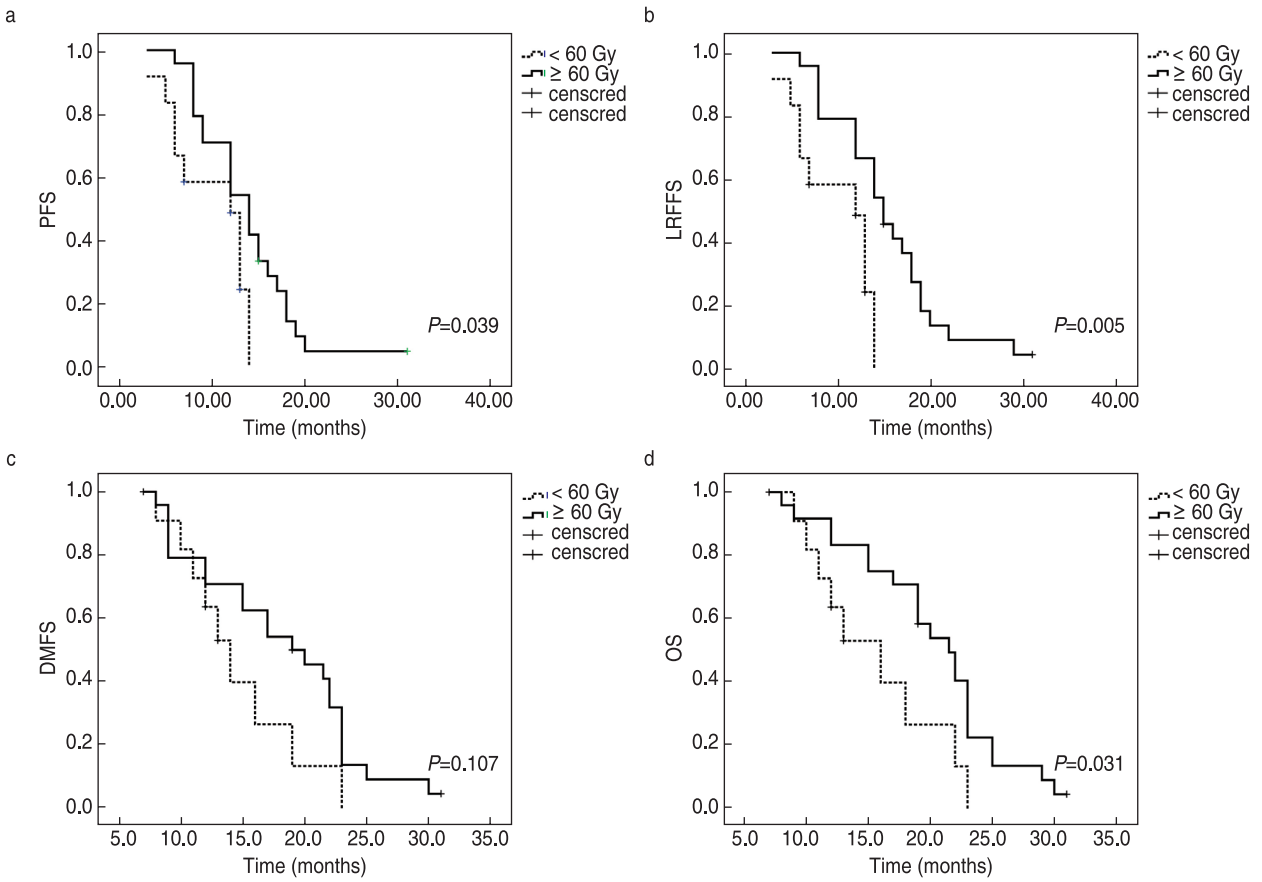

Fig. 2 Survival curves of progression-free survival, loco-regional failure-free survival, distant metastasis-free survival, and overall survival stratified according to radiation dose

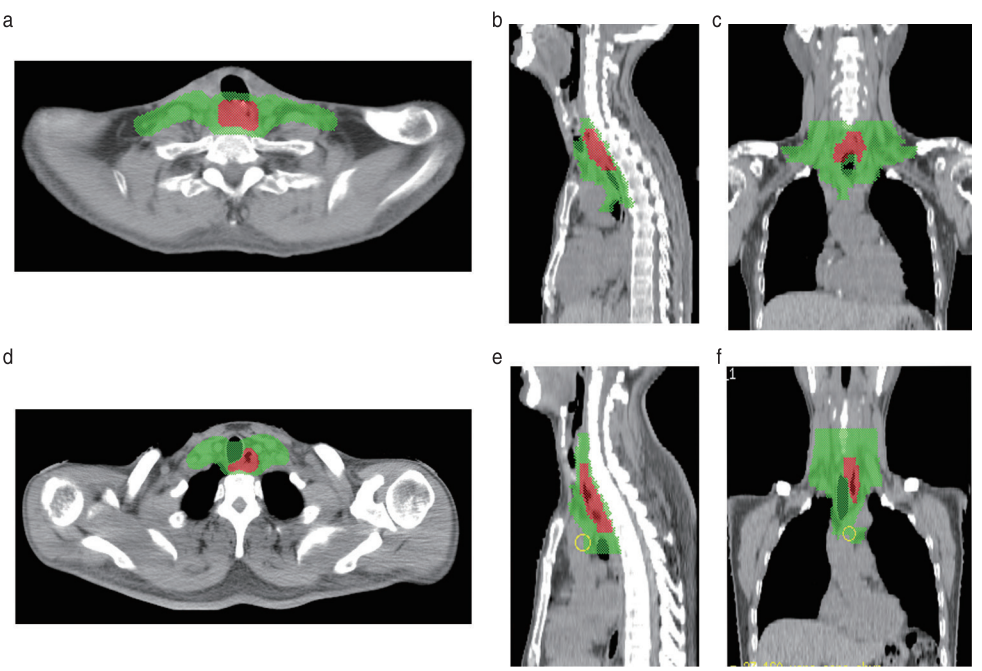

Fig. 3 Gross tumor volume (red area) and clinical tumor volume (green area) for proximal cervical esophageal cancer (CEC) (a–c) and distal CEC (d–f) in patients who received intensity-modulated radiotherapy. a and d, transverse position; b and e, sagittal position; c and f, coronal position

Radiation dose had a slight significant association with poor PFS (P = 0.081). As the cutoff value of the univariate analysis was set to P < 0.01, a multivariate analysis of the association between hoarseness and radiotherapy dose as well as PFS was conducted. Results showed that hoarseness, but not radiation dose, was significantly correlated with PFS (P = 0.040 and 0.115, respectively) (Table 3).

However, in the subgroup analysis, hoarseness was not significantly associated with survival outcome. In the proximal CEC subgroup, four patients presented with hoarseness (PFS, P = 0.341; LRFFS, P = 0.166; DMFS, P = 0.371; and OS, P = 0.229). Meanwhile, in the distal CEC subgroup, eight patients with hoarseness (PFS, P = 0.157; LRFFS, P = 0.097; DMFS, P = 0.055; and OS, P = 0.053).

Treatment failure patterns

In total, 53 patients experienced treatment failure during the follow-up period. Of them, 42 (79.2%) presented with locoregional failure and 14 (22.22%) with distant failure. In patients who received a radiation dose < 60 Gy, 19 (70.37%) developed locoregional failure. Meanwhile, in patients who received a radiation dose ≥ 60 Gy, 23 (63.89%) experienced locoregional failure. The Pearson's chi-square test revealed no significant difference (χ^2 = 0.292; P = 0.589) between the two groups. In the proximal CEC subgroup, 9 (75.0%) and 15 (62.5%) patients who received radiation doses < 60 and ≥ 60 Gy, respectively, developed locoregional failure. However, the result was not significantly different (χ^2 = 0.562; p = 0.453). In the distal CEC subgroup, 11 (73.33%) and 7 (58.33%) patients who received radiation doses < 60 and ≥ 60 Gy, respectively, developed locoregional failure. However, the result was not significantly different (χ^2 = 0.675; P = 0.411).

Toxicities

There were no treatment-related deaths. The most common grade 1 or 2 acute toxicities were mucositis, skin reactions, and hemocytopenia. There was no significant difference in terms of ≥ grade 3 acute toxicities between the groups who received radiation doses < 60 and ≥ 60 Gy (Table 4). In terms of late toxicities, the incidence of esophageal stenosis was similar between the two groups (7.41% *vs* 8.33%, p = 0.893). Moreover, the incidence of tracheoesophageal fistula was higher in the group who received a radiation dose ≥ 60 Gy group than in the group who received a radiation dose < 60 Gy. However, the difference was not significant (13.89% *vs* 7.41%, p = 0.418) (Table 4). Notably, only one patient who received a radiation dose of 70 Gy presented with grade 4 esophageal stenosis.

Discussion

Due to the low incidence of CEC, clinical data on the survival outcomes of definitive radiotherapy are limited. In particular, the number of studies that used modern radiation techniques is extremely low. In the current study, 63 CEC patients, most of whom (56/63, 88.9%) received IMRT, were included. Results showed that there was no significant difference in terms of survival between the groups who received GTV radiation doses ≥ 60 and < 60 Gy. According to the location of the primary tumor above or below the middle portion of the cervical esophagus, the patients were further divided into the proximal and distal CEC subgroups. In the proximal CEC subgroup, the PFS, OS, and LRFFS significantly improved in patients who received a GTV radiation dose ≥ 60 Gy compared with those who received a GTV radiation dose < 60 Gy. Meanwhile, in the distal CEC subgroup, the PFS, OS, and LRFFS did not significantly improve. Univariate and multivariate analyses revealed that hoarseness was the only independent prognostic factor of survival among patients with CEC. No significant difference was observed in terms of the occurrence of severe toxicities.

To date, the largest series, which included 789 CEC

Table 2 Univariate analysis of prognostic factors influencing PFS, OS, LRFFS and DMFS in CEC

Factors	PFS		OS		LRFFS		DMFS	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Gender (Male *vs* Female)	0.793 (0.425-1.481)	0.467	0.663 (0.351-1.251)	0.204	0.585 (0.311-1.102)	0.097	0.872 (0.464-1.639)	0.671
Age (< 60 years *vs* ≥ 60 years)	0.700 (0.408-1.202)	0.196	0.879 (0.509-1.518)	0.644	0.817 (0.477-1.399)	0.461	0.885 (0.517-1.514)	0.655
Fistula	0.790 (0.397-1.571)	0.502	0.753 (0.377-1.504)	0.422	0.587 (0.293-1.176)	0.133	0.901 (0.453-1.791)	0.765
NLR (< 4 *vs* ≥ 4)	0.795 (0.417-1.515)	0.485	0.928 (0.478-1.803)	0.825	0.862 (0.453-1.639)	0.650	0.806 (0.425-1.530)	0.510
Hoarseness	0.506 (0.264-0.968)	0.040	0.398 (0.202-0.786)	0.008	0.503 (0.265-0.953)	0.035	0.452 (0.232-0.879)	0.019
T classifi-cation (T1-2 *vs* T3-4)	0.793 (0.417-1.506)	0.478	0.876 (0.460-1.668)	0.686	0.672 (0.353-1.279)	0.226	0.981 (0.516-1.864)	0.952
N classifi-cation (N0 *vs* N+)	0.739 (0.426-1.281)	0.281	0.710 (0.404-1.247)	0.233	0.751 (0.427-1.318)	0.318	0.754 (0.435-1.307)	0.314
TNM stage (1-2 *vs* 3-4)	0.873 (0.507-1.503)	0.624	0.751 (0.430-1.312)	0.314	0.837 (0.479-1.462)	0.532	0.808 (0.470-1.390)	0.442
RT Dose (< 60 Gy *vs* ≥ 60 Gy)	1.643 (0.941-2.869)	0.081	1.310 (0.758-2.265)	0.334	1.396 (0.813-2.398)	0.227	1.401 (0.822-2.388)	0.216

P < 0.05 was considered to indicate a statistically significant difference. PFS, progression free survival; OS, overall survival; LRFFS, loco-regional failure-free survival; DMFS, distance metastasis free survival; CEC, cervical esophageal carcinoma; HR, hazard ratio; CI, confidence interval; NLR, neutrophil to lymphocyte ratio; RT, radiotherapy

Table 3 Multivariate analysis of prognostic factors influencing PFS in CEC

Endpoint	Variable	HR	95% CI	*P* value
PFS	Hoarseness	0.506	0.264-0.968	0.040
	RT Dose	1.561	0.893-2.731	0.115

P < 0.05 was considered to indicate a statistically significant difference. PFS, progression free survival; CEC, cervical esophageal carcinoma; RT, radiotherapy; HR, hazard ratio; CI, confidence interval

Table 4 Toxicities ≥ grade 3 in CEC patients received definitive radiotherapy stratified by radiation dose

Toxicities ≥ Grade 3	Dose < 60 Gy *n* = 27	Dose ≥ 60 Gy *n* = 36	*P* value
Acute toxicity			
Dysphagia	2 (7.41%)	4 (11.11%)	0.620
Skin	0 (0%)	1 (2.78%)	0.383
Mucositis	2 (7.41%)	1 (2.78%)	0.393
Hemocytopenia	1 (3.70%)	2 (5.56%)	0.733
Chronic Dysphagia			
Esophageal stenosis	2 (7.41%)	3 (8.33%)	0.893
Tracheoesophageal fistula	2 (7.41%)	5 (13.89%)	0.418

P < 0.05 was considered to indicate a statistically significant difference. CEC, cervical esophageal carcinoma

patients from the National Cancer Data Base (NCDB), reported that compared with standard-dose radiation (50–50.4 Gy), medium-dose (50.4–66 Gy) or high-dose (66–74 Gy) radiation could not significantly improve OS [14]. This result was consistent with that of the INT-0123/RTOG 94-05 trial8 and other studies [15]. However, there are still controversies regarding this finding. Even in the NCDB analysis, from 2004 to 2013, 73% of CEC patients were treated with radiation doses > 50.4 Gy, indicating that most oncologists support dose escalation for CEC. One prospective clinical trial on CEC in Japan, which included 30 patients, used radiation therapy with 3D CRT at a dose of 60 Gy in 30 fractions [16]. Recently, Herrmann *et al* reported that high-dose (> 56 Gy) radiation was significantly correlated with better DFS and OS in proximal esophageal carcinoma. Moreover, Wang *et al* showed that a radiation dose > 50 Gy significantly increased the rate of complete response and OS in patients with cervical and upper thoracic esophageal cancer from the MD Anderson Cancer Center [12]. A study in Canada conducted a retrospective analysis of 81 CEC patients who received consecutive treatment based on three protocols (protocol 1: two-dimensional radiation (2D RT) of 54 Gy in 20 fractions with 5-Fu plus mitomycin C/cisplatin; protocol 2: 3D CRT ≥ 60 Gy in 30 fractions with ENI plus cisplatin; and protocol 3: IMRT ≥ 60 Gy in 30 fractions with ENI plus cisplatin). Results showed that the patients treated with protocol 3 had better OS than those treated with protocol 1, with benefits similar to those of protocol 2 [17].

The contrasting results are partly attributed to the use of different radiation techniques. In the RTOG 94-05 trial, 2D RT was used, which increased the incidence of radiation toxicities in normal tissues when the dose reached 64.8 Gy. Only 67% of patients completed the radiation therapy in the group who received a dose of 64.8 Gy and 83% in the group who received a dose of 50.4 Gy. Moreover, there were 11 treatment-related deaths in the high dose arm and 2 in the 50.4 Gy arm, which was one of the main causes of treatment failure. Therefore, high-dose radiation using old techniques results in severe side effects, which might compromise the benefit of high-dose therapy on tumors. However, the modern photon-based radiotherapy techniques, such as 3D CRT, IMRT and volumetric-modulated arc therapy, can have high conformity to the target volume, which concurrently facilitates the delivery of higher doses to tumors and the sparing of adjacent normal organs at risk. Retrospective studies showed that IMRT could improve local-regional control and OS among CEC patients [17–18]. Thus, more data about the efficacy of high-dose radiation using modern techniques in CEC must be collected to help direct clinical treatment.

In the current study, of 63 patients, 34 (60.7%) and 22 (39.3%) received radiation doses ≥ 60 Gy and < 60 Gy, respectively. Moreover, 56 (88.9%) patients received IMRT and seven 3D CRT. However, high-dose radiation did not significantly improve survival compared with standard-dose radiation. However, a similar trend was observed for PFS ($P = 0.053$). The biological behaviors of tumor usually differ due to location, such as left and right side of the colon. Hence, we further divided the patients into the proximal and distal CEC subgroups according to the location of the primary tumor above or below the middle portion of the cervical esophagus. Notably, in the proximal CEC subgroup, high-dose radiation was significantly correlated with better PFS, LRFFS, and OS. Meanwhile, the difference was not significant in the distal CEC subgroup. Results showed that proximal CEC might be more analogous to hypopharyngeal carcinoma, and high-dose radiation (≥ 60 Gy) could improve the prognosis of patients. However, distal CEC might be more analogous to thoracic esophageal carcinoma. Kim *et al* reported that compared with the thoracic esophagus, the cervical esophagus could receive high-dose radiation because only a small portion of the lungs is irradiated during radiotherapy [11]. Similar with this standpoint, the proximal CEC subgroup in our study could tolerate a higher radiation dose compared with the distal CEC subgroup, as the primary tumor is mainly located in the cervical esophagus with less extension to the thoracic esophagus. Thus, high-dose radiation is more advantageous in proximal CEC than in distal CEC.

Considering an abundant lymphatic drainage,

metastasis to regional lymph nodes is common in CEC. However, the delineation of CTV, mainly regarding the need for ENI, still remains controversial. Hirano *et al* reported that in hypopharyngeal carcinoma and CEC, the incidence rates of cervical (levels II–IV) and upper mediastinal lymph nodal metastasis were 85.7% and 33.3%, respectively [19]. Moreover, 0% to 25% patients treated with dCCRT or dRT developed regional lymph node recurrence with ENI versus at least 25% without ENI [20–22]. The NCCN guidelines recommend that prophylactic radiation of cervical and supraclavicular nodes should be considered particularly if the nodal classification is N1 or greater [5]. Conversely, some studies do not recommend ENI because a wide radiation field might aggregate toxicity and interrupt or even terminate dCCRT and does not improve survival [4, 23]. A recent study showed that ENI might destruct lymphocytes in the nearby lymph nodes and affect immune response due to radiation, which were critical for tumor control [24] In our study, 51 (81.0%) of 63 patients, including 12 patients with N negative, received ENI according to the physician's discretion.

In previous studies, CEC patients received definitive radiotherapy, and the 3-year OS rate ranged from less than 35% to nearly 40% [7, 13, 25]. However, in this study, the median OS was only 19 months, which is relatively poor. One of the main causes of this outcome was advanced disease stage. That is, 48 (76.2%) patients with stage T3–4 disease, 35 (55.5%) with stage III disease, and 3 with stage IV disease with bone metastasis at a stable single site. These patients could also benefit from definitive radiotherapy. Another reason is that 33.3% of patients were treated without concurrent chemotherapy due to poor performance or intolerance to CCRT, which might reduce disease control and survival.

An analysis revealed that locoregional failure was still the main pattern of failure in CEC patients. Moreover, even in proximal CEC, the difference in locoregional control rate was not significant between the groups who received GTV radiation doses ≥ 60 Gy and < 60 Gy. This result indicated that high-dose radiation (≥ 60 Gy) might only delay, but not prevent, the occurrence of locoregional failure.

In addition, hoarseness, which is caused by tumor invasion or compression of the recurrent laryngeal nerve, was considered an independent prognostic factor of survival in patients with CEC. However, in a subgroup analysis (proximal and distal CEC subgroups), hoarseness did not significantly affect survival outcomes, which might be attributed to the limited number of patients included in the study. Moreover, other studies showed the prognostic role of hoarseness in CEC [25], which must be considered in clinical settings.

The current study had several limitations due its retrospective nature. First, 63 patients were included in the study, which is relatively small. Moreover, the data used were from a single institution, and this might have affected the reliability of the findings. Second, potential confounding factors, including different characteristics (such as clinical stage, concurrent chemotherapy, and ununiform chemotherapy regimens), might also limit the applicability of the conclusion. However, since CEC is a rare disease, large-scale prospective multicenter randomized control trials are challenging to perform. Thus, retrospective studies are essential in obtaining evidence for clinical treatment.

In conclusion, for proximal CEC, a GTV radiation dose ≥ 60 Gy was significantly correlated with better PFS, OS, and LRFFS. Moreover, the survival of patients with whole and distal CEC did not significantly improve. However, the treatment-related toxicities were acceptable. Proximal and distal CEC might have distinct biological behaviors, which are important in the selection of clinical treatment. Proximal CEC was more analogous than hypopharyngeal carcinoma, and patients with this condition could benefit from high-dose radiation (≥ 60 Gy). Meanwhile, distal CEC was more analogous than thoracic esophageal carcinoma. Thus, high-dose radiation was not beneficial for patients with distal CEC. Further prospective randomized controlled clinical trials must be conducted to validate the results of the current study. Considering the opportunities and challenges of radiotherapy [26], in the future, optimal CCRT regimens, radiosensitizing agents, and new therapeutic targets must be developed to improve the survival outcomes of CEC patients.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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ORIGINAL ARTICLE

Neurotrophin 3 hinders the growth and metastasis of hepatocellular carcinoma cells*

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Abstract

Objective Neurotrophin 3 (*NTF3*) is involved in numerous biological processes; however, its role in hepatocellular carcinoma (HCC) is not well studied. This study investigated *NTF3* function in HCC progression and revealed its underlying molecular mechanisms.

Methods The prognostic relevance of *NTF3* was determined through a bioinformatical analysis of publicly available TCGA data. Immunohistochemistry of HCC biopsies was performed to explore the expression of *NTF3*. Cell growth and proliferation were analyzed using a Cell Counting Kit-8 (CCK-8) assay. Cell invasion and migration were analyzed using Boyden Transwell and wound healing assays. Protein expression and mRNA levels were evaluated through immunoblotting and quantitative polymerase chain reaction (qPCR). Cell apoptosis was evaluated with flow cytometry.

Results *NTF3* expression was significantly lower in HCC tissues than in adjacent non-tumor tissues. Low *NTF3* expression was significantly associated with decreased patient survival and specific clinicopathological features. *NTF3* overexpression reduced the proliferation, migration, and invasion abilities of HCC cell lines.

Conclusion Decreased expression of *NTF3* is associated with poor prognosis in HCC patients, likely due to its action in promoting HCC cell proliferation, migration, and invasion. Our findings provide a novel understanding into the pathogenesis of HCC and the role of *NTF3* in tumor progression, suggesting that targeting *NTF3* has potential therapeutic and diagnostic value for HCC.

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Key words: hepatocellular carcinoma; tumor progression; neurotrophin 3 (NTF3)

Hepatocellular carcinoma (HCC) is a highly lethal cancer with a rapidly increasing worldwide incidence. HCC accounts for 75%–85% of primary liver cancer cases [1–5]. Surgical treatment is one of the main forms of treatment for liver cancer, but chemotherapy, targeted therapy and immunotherapy will likely be recommended in the near future as non-invasive approaches [6–9]. However, early biomarkers and tumor-specific treatments for HCC are limited. A deeper understanding of the pathogenesis of HCC will be instrumental for early detection and treatment of the disease [10–11], which is why it is so important to find early diagnostic markers and novel therapeutic targets.

Neurotrophin-3 (*NTF3*) is a member of the neurotrophin family that includes nerve growth factor, brain-derived neurotrophic factors, and neurotrophin 4/5. *NTF3* is a growth factor that is involved in stem cell differentiation into neuron-like cells [12]. Previous studies of *NTF3* have focused on neuronal differentiation, osteoarthritic cartilage, neurogenesis, neural survival and Alzheimer's disease (AD) [12–18]. Research on *NTF3* in the field of cancer is rare and is limited to its role in breast cancer [19]. Previous studies have shown that in triple-negative breast cancer, *NTF3* is capable of activating TrkB to induce anoikis resistance [19].

Interestingly, our bioinformatics analysis indicated that *NTF3* may be involved in the development of HCC as a tumor suppressor gene. HCC has a very complex

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molecular pathogenesis and the underlying molecular mechanisms that initiate HCC involve several critical signaling pathways that promote the carcinogenic process [20–21]. There is evidence of enhanced anoikis-suppression through activation of the PI3K/Akt/Bcl-2 pathway in HCC cells [22]. Thus, we speculated that *NTF3* may exert an anti-tumor effect by inducing apoptosis of cancer cells.

This study aimed to explore the role of *NTF3* in the development of HCC and how it regulates this process. This study provides new insights into the molecular mechanisms underlying HCC progression and provides a new therapeutic target for HCC.

Materials and methods

Samples and informed consent

In total, 80 pairs of HCC and corresponding adjacent tissues (from areas in the vicinity (< 2 cm) of the tumor tissue with distinctly different edges) were obtained during surgical resections of patients without preoperative treatment at The Affiliated Hospital of Qingdao University (Qingdao, China). Human specimen collection was conducted in accordance with the guidelines of the Medical Ethics Committee of Affiliated Hospital of Qingdao University and approved by the Affiliated Hospital of Qingdao University Joint Institutional Review Board. All donors provided informed written consent prior to specimen collection according to the policies of the committee. The resected samples were identified by two pathologists independently.

Cell culture

In this study, one healthy liver cell line (HL-7702) and four HCC cell lines (SMMC-7721, Huh-7, BEL-7402 and HCCLM9) were used. All cell lines were obtained from the Cell Resource Center of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China) and cells were authenticated, tested for mycoplasma infection, and cultured in a humidified atmosphere of 5% CO_2 at 37 °C in Dulbecco's Modified Eagle medium (DMEM) medium containing 10% fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA) and 1% penicillin and streptomycin. The medium was replaced every 2 days. Cells were monitored using microscopy to ensure that they maintained their original morphology.

Plasmid transfection

NTF3 overexpression plasmid constructs, *NTF3* shRNA, and their corresponding controls were provided by GeneChem. The plasmid overexpressing *NTF3* was transfected into SMMC-7721 cells, while the RNA-interference-treated *NTF3* plasmid was transfected into Huh7 cells. Endogenous *NTF3* expression was detected using real-time PCR after 24 h.

For the transfection experiments, cells were seeded in 24-well plates and incubated overnight at 37 °C with 5% CO_2. The above plasmids were transfected into the cells using Lip3000 (Life Technologies) according to the manufacturer's protocol. The HCC cells were then cultured for 24 h at 37 °C with 5% CO_2.

Wound-healing assays

For wound healing assays, cells were seeded into a 6-well plate and cultured at 37 °C for 24 h. Wounds were created in monolayers of cells using a 200 μL pipette tip. Cells were washed to remove cellular debris and incubated in DMEM without FBS at 37 °C with 5% CO_2. Images were taken at 0 h and 24 h after wounding. The wound area was measured and the percentage of wound healing was calculated using Image J software (NIH, Bethesda, MD, USA). This experiment was repeated three times.

CCK-8 assays

Cell proliferation was measured using the CCK-8 (Dojindo, Kumamoto, Japan). Briefly, the cell density of the treated cells was adjusted to 5×10^4 cells/mL with DMEM. Cells were then inoculated in a 96-well plate with 100 μL of cell suspension per well and incubated at 37 °C overnight. After culturing, the cells were washed and 10 μL of CCK-8 solution was added into each well of the plate. Cells were subsequently incubated for 4 h at 37 °C with 5% CO_2, and the absorbance was measured at 450 nm with a microplate spectrophotometer.

Transwell invasion assays

Cell invasion was measured using Matrigel-coated Transwell cell culture chambers. Cells in the logarithmic phase were starved in serum-free medium for 24 h, after which they were digested using 0.25% EDTA-trypsin. The cell suspension was then treated with serum-free medium, during which the density of the suspension was adjusted to 2×10^5/mL. Then, 100 μL of Matrigel with a final concentration of 1 mg/ml was added to the bottom of the upper chamber followed by incubation for 4–5 h at 37 °C to make it gelatinous. After the Matrigel was gelatinized, wells in the Transwell chamber were connected. The cell suspension was cultured in a 37 °C, 5% CO_2 incubator for 24 h. Three duplicate systems were used for each group. After 24 h, the chamber was dislodged, carefully cleansed once with PBS, and the cells were fixed with 70% ethanol for 1 h, then dried at room temperature. The chamber was then dyed with 0.5% crystal violet for 20 min, washed with PBS, and the upper side of the chamber was cleansed with a clean cotton ball. The migrated cells were wiped and cleaned, and the chamber was placed under an inverted microscope so that the remaining cells could be counted. The images were

analyzed using ImageJ software.

Apoptosis analysis

A total of 1×10^6 cells were cultured overnight and collected by trypsin digestion. The cells were washed with PBS followed by subsequent incubation at room temperature in the dark for 15 min, according to the manufacturer's protocol (AnnexinV-APC/7-AAD). Cell apoptosis was detected using a flow cytometer (BD Biosciences, USA).

Quantitative reverse transcription-PCR

Total RNA from tissues was extracted using Trizol (Takara) according to the instructions provided by the manufacturer and was treated with recombinant DNase I (RNase-free) (Code No. 2270A). Removal of genomic DNA was performed using gDNA Eraser. Reverse transcription was performed with 1 μg of RNA using RT Primer Mix mixed with Random 6 mers and Oligo dT Primer. Quantitative RT-PCR was performed using TB Green Premix (Takara, Otsu, Japan) in a LightCycler® 96 SW 1.1 machine (Roche). All reactions were performed in triplicate and GAPDH was used as internal control. The data were analyzed using the delta Ct method. Specific primer sequences for qRT-PCR were: NTF3-F (Forward): ATGATAAACACTGGAACTCT, NTF3-R (reverse): TAT CCGTATCCACCGCCAGC; GAPDH-F: TCATGGGTGT GAACCATGAGAA, GAPDH-R: GGCATGGACTGTGGT-CATGAG.

Western blot

Cells were scraped into RIPA buffer containing protease and phosphatase inhibitors. Extracted proteins were separated in 10% SDS-PAGE gels (Bio-Rad, 4561095) and transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad). Membranes were blocked in 5% skim milk powder in Tris-buffered saline (TBS) with Tween 20 (TBS-T) for 2 h at room temperature. Incubation with primary antibodies was performed at 4 °C overnight. Membranes were washed with TBST, incubated with peroxidase-conjugated secondary antibody for 2 h and developed using the Enhanced Chemiluminescence (ECL) Detection System (Thermo Scientific). Antibodies were as follows: GAPDH (Cell Signaling #5174S, 1:1000), *NTF3* (abcam #Ab53685, 1:500), Caspase 3 (Cell Signaling #14220S, 1:1000), Bax (abcam #Ab32503, 1:1000), Bcl2 (Cell Signaling #3498S, 1:1000).

Statistical analysis

Statistical analysis was performed using the SPSS program (version 18.0; SPSS, Chicago, IL, USA). Data are presented as mean ± SD. Statistical significance was calculated using Student's *t*-test, χ^2 test, Fisher's exact test or one-way ANOVA. Pearson's analysis was used in correlation analyses. $P < 0.05$ was considered as statistically significant.

Results

NTF3 expression is decreased in HCC tissues and correlates with clinico-pathological characteristics

To identify genes with crucial roles in liver tumorigenesis, we first analyzed publicly available gene expression data from The Cancer Genome Atlas (TCGA), and screened genes that were differentially expressed in HCC tissues compared to normal tissues. As shown in Fig. 1a, several genes were identified with decrease or increased expression in HCC. *NTF3* was identified among the downregulated genes as having a potential association with liver tumorigenesis. A boxplot showing the expression of *NTF3* in HCC tissue compared with normal tissue is depicted in Fig. 1b. Survival analysis from the TCGA database indicated that low expression of *NTF3* was associated with poor survival of HCC patients while high expression of *NTF3* was associated with prolonged patient survival (Fig. 1c).

To experimentally verify the *in-silico* data, we collected clinical samples and assessed *NTF3* mRNA levels in HCC tissues and ANLTs by qRT-PCR. Consistent with the bioinformatical analysis results, the expression level of *NTF3* in HCC tissues was reduced compared to ANLTs (Fig. 2a). Immunohistochemistry was also performed on tumors and adjacent tissues collected from patients. Representative images of *NTF3* staining in the HCC and paracancerous tissue samples are shown in Fig. 2b. Negative *NTF3* staining was observed in HCC tissues, while normal adjacent tissues were positive for *NTF3* expression. Together, these results indicate that *NTF3* mRNA and protein expression is decreased in HCC tissues *in vivo*.

To explore the correlation between *NTF3* and clinicopathological variables, HCC tissues were divided into two groups: high and low *NTF3*-expression groups. Then, *NTF3* expression was correlated with clinicopathologic characteristics of HCC. The results (Table 1) indicated that gender, age, AFP and HBsAg were not associated with *NTF3* expression levels. In contrast, tumor size, the number of tumors present, tumor differentiation level, chronic hepatitis, liver cirrhosis, vascular invasion, invasion of nerves, TNM stage and BCLC were all inversely associated with *NTF3* expression. These observations indicate that decreased expression of *NTF3* is associated with tumor progression.

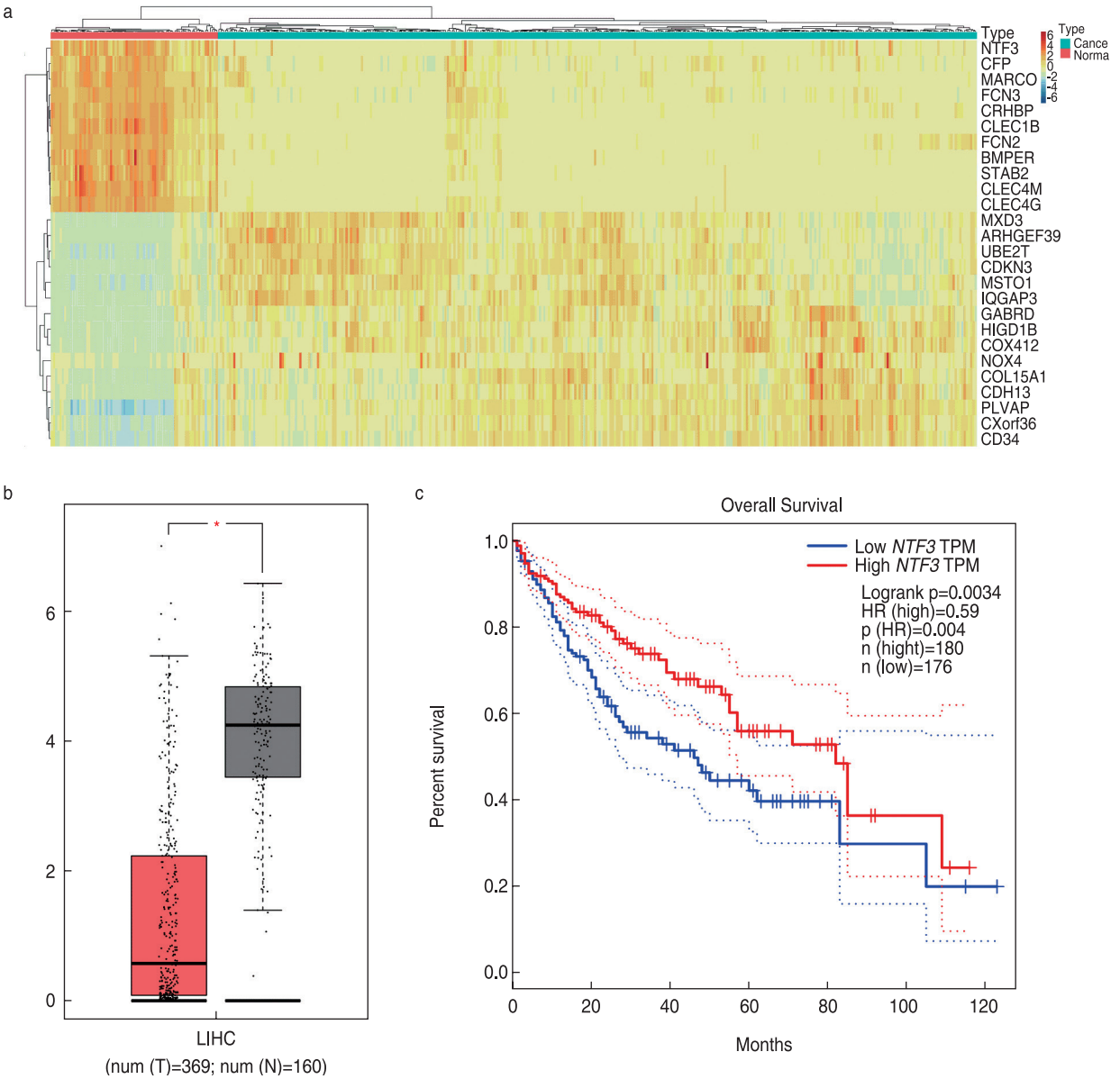

Fig. 1 Analysis of hepatocellular carcinoma (TCGA-LIHC). (a) Heatmap showing differentially expressed genes in HCC tumors and healthy samples; (b) Box plot showing the expression of *NTF3* in HCC tumors and healthy samples; (c) Effect of *NTF3* expression on the survival of LIHC patients

Expression of NTF3 in vitro and transfection efficiency analysis.

To explore the biological function of *NTF3*, *in vitro* experiments were performed using HCC cell lines. First, we measured the mRNA expression levels of *NTF3* in four HCC cell lines and healthy human hepatocytes using qRT-PCR. The results (Fig. 2c) indicated that *NTF3* expression was significantly decreased in the four HCC cell lines (Huh7, SMMC-7721, HCCLM9 and BEL-7402) compared to the healthy hepatocytes HL-7702. Among the four HCC cell lines, SMMC-7721 cells had the lowest average *NTF3* mRNA expression while Huh7 cells had the highest expression level. Thus, for functional analysis, *NTF3* was overexpressed in SMMC-7721 cells through cell transfection and silenced in Huh7 cells using *NTF3* shRNAs. The expression efficiency after shRNA silencing and overexpression are shown in Figure 2D and 2E, respectively. Transfection with the overexpression plasmid effectively and significantly increased the expression of *NTF3*, while transfection with three shRNAs markedly decreased *NTF3* expression.

Table 1 Correlation between the clinicopathologic chatacteristics and NTF3 expression in HCC tissues [*n* (%)]

Characteristics	Total number of patients (*n* = 80)	No. of patients		*P* value
		$NTF3^{low}$ *n* = 63	$NTF3^{high}$ *n* = 17	
Gender				
Male	66 (82.5)	52 (82.5)	14 (82.4)	1.0[b]
Female	14 (17.5)	11 (17.5)	3 (17.6)	
Age (years)				
≤ 60	49 (61.3)	37 (58.7)	12 (70.6)	0.79[a]
> 60	31 (38.8)	26 (41.3)	5 (29.4)	
Tumor size(cm)				
≤ 3	30 (37.5)	17 (27.0)	13 (76.5)	< 0.001†[a]
> 3	50 (62.5)	46 (73.0)	4 (23.5)	
Number of tumors				
1	38 (47.5)	25 (39.7)	13 (76.5)	0.0070†[a]
≥ 2	42 (52.5)	38 (60.3)	4 (23.5)	
Tumor differentiation level				
I–II	22 (27.8)	10 (15.9)	12 (70.6)	< 0.001†[b]
III–IV	57 (72.2)	53 (84.1)	5 (29.4)	
AFP (ng/mL)				
≤ 20	42 (52.5)	30 (47.6)	12 (70.6)	0.092[a]
> 20	38 (47.5)	33 (52.4)	5 (29.4)	
Chronic hepatitis				
No	21 (26.2)	8 (12.7)	13 (76.5)	< 0.001†[a]
Yes	59 (73.8)	55 (87.3)	4 (23.5)	
HBsAg				
Absent	46 (57.5)	38 (60.3)	8 (47.1)	0.33[a]
Present	34 (42.5)	25 (39.7)	9 (52.9)	
Liver cirrhosis				
Absent	21 (26.2)	10 (15.9)	11 (64.7)	< 0.001†[b]
Present	59 (73.8)	53 (84.1)	6 (35.3)	
Vascular invasion				
No	18 (22.5)	3 (4.8)	15 (88.2)	< 0.001†[b]
Yes	62 (77.5)	60 (95.2)	2 (11.8)	
Invasion of nerves				
No	39 (48.8)	22 (34.9)	17 (100.0)	< 0.001†[b]
Yes	41 (51.2)	41 (65.1)	0 (0.0)	
TNM				
I	15 (18.8)	4 (6.3)	11 (64.7)	< 0.001†[b]
I–III	65 (81.2)	59 (93.7)	6 (35.3)	< 0.001†[b]
IV	0 (0.0)	0 (0.0)	0 (0.0)	
BCLC				
0–A	30 (37.5)	20 (31.7)	10 (58.8)	0.041†[a]
B–C	11 (13.8)	6 (9.5)	5 (29.4)	0.0496†[b]
D	39 (48.8)	37 (58.7)	2 (11.8)	< 0.001†[a]
Child level				
A	19 (23.8)	5 (7.9)	14 (82.4)	< 0.001†[b]
B	22 (27.5)	20 (31.7)	2 (11.8)	0.13[b]
C	39 (48.8)	38 (60.3)	1 (5.9)	< 0.001†[a]

[a] Peason chi-squared test; [b] Fischer's exact test; † Bold text indicates statistical significance (*P* < 0.05)

NTF3 hinders HCC cell proliferation and promotes apoptosis in vitro

Cell Counting Kit-8 (CCK-8) assays were performed to assess the role of *NTF3* in the proliferation of HCC cells. Compared with the untransfected group and the negative control (NC) group, overexpression of *NTF3* in SMMC-7721 cells significantly decreased cell viability (Fig. 3a). In contrast, the rate of apoptosis in SMMC-7721 cells was markedly increased following *NTF3* overexpression (Fig. 3b and 3c). In addition, we found that silencing *NTF3* in Huh7 cells significantly promoted cell proliferation compared to the untransfected and NC groups (Fig. 3d). Moreover, flow cytometry analysis of cell apoptosis indicated that the rate of apoptosis in Huh7 cells was decreased after *NTF3* silencing (Fig. 3e and 3f).

To further confirm the effect of *NTF3* on cell apoptosis, Western blot analysis was performed on SMMC-7721 cells overexpressing *NTF3* and Huh7 cells in which *NTF3* was silenced. *NTF3* overexpression significantly increased the expression of cleaved-caspase 3 and Bax but decreased the expression of Bcl2 in SMMC-7721 cells (Fig. 4a, 4b, 4d, and 4e). Furthermore, silencing of *NTF3* caused a decrease in the expression of cleaved-caspase 3 and Bax, but increased the expression of Bcl2 in Huh7 cells (Fig. 4a, 4c, 4d, and 4f). These results indicate that *NTF3* hinders HCC cell proliferation and induces apoptosis *in vitro*.

NTF3 hinders HCC cell migration and invasion in vitro

Transwell and wound healing assays were carried out to explore the effects of *NTF3* on HCC cell migration. The Transwell assay indicated that overexpression of *NTF3* could inhibit the invasive activity of SMMC-7721 cells while knockdown of *NTF3* could promote the invasive activity of Huh7 cells (Fig. 5a and 5c). The wound healing assay showed that the wound closure of SMMC-7721 cells overexpressing *NTF3* proceeded slower than that of the untransfected and NC groups (Fig. 5b), whereas suppression of *NTF3* expression in Huh7 cells resulted in faster wound closure compared to the two control groups (Fig. 5d). These data suggest that *NTF3* can inhibit the metastasis of HCC cells *in vitro*.

Discussion

Regardless of the efforts made in anti-cancer research, patients with HCC still have a poor prognosis [23–26]. To uncover effective biomarkers for improving the diagnosis and prognosis of HCC, we examined the functions of *NTF3* in HCC *in vivo* and *in vitro*. We determined the expression level and the role of *NTF3* in HCC cells using

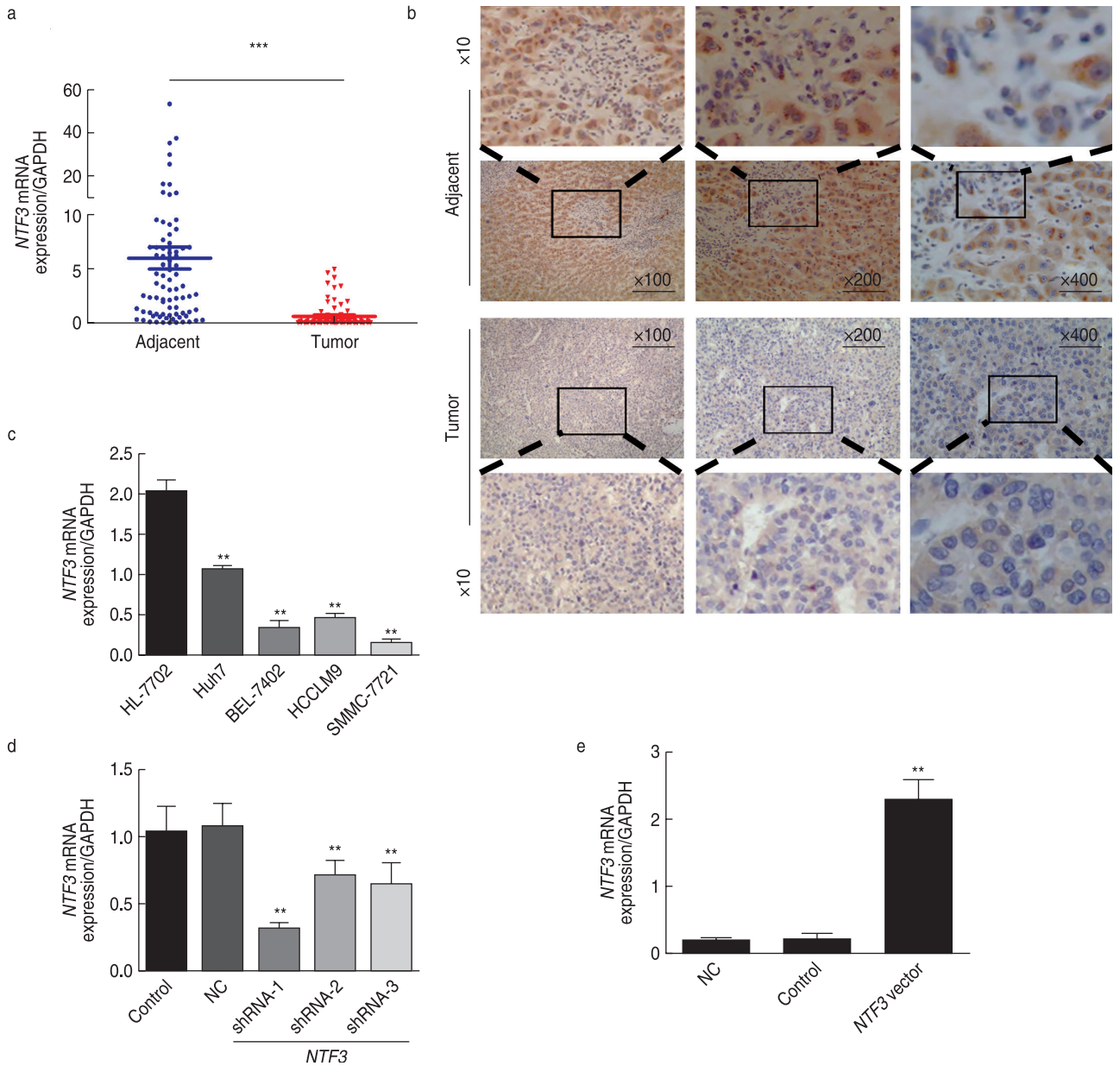

Fig. 2 *NTF3* is downregulated in HCC. (a) Box plot showing the expression of *NTF3* in HCC tumors and adjacent tissues; (b) Immunohistochemistry showing the expression of *NTF3* in tumors and adjacent tissues; (c) NTF3 expression in HCC cells; (d) *NTF3* mRNA expression in Huh7 cells following *NTF3* shRNA transfection; (e) *NTF3* mRNA expression in SMMC-7721 cells following transfection of an *NTF3* expression vector

different approaches. We found that upregulation of *NTF3* is strongly associated with decreased overall TNM stage and longer survival times. These results implicate *NTF3* in HCC pathogenesis and suggest its low expression is associated with the progression and metastasis of HCC. Moreover, our study proposed the targeting of *NTF3* as a potential treatment for HCC in addition to its possible use as a predictive marker of HCC outcomes in patients.

NTF3 has been suggested as a therapeutic target for breast cancer therapy [19]. Indeed, *NTF3* expression is increased in brain metastatic breast cancer cells and it has been demonstrated to promote the proliferation and metastasis of breast cancer cells in the brain by promoting the re-epithelialization of these cells and downregulating the microglial cytotoxic response [19]. In the present study, our results did not corroborate with these previous finding as we found that *NTF3* expression was decreased in HCC tissues and cells through different technical approaches (immunohistochemistry, Western blotting and qRT-PCR). These contradictory results may be due to the neuroprotective role of *NTF3* in the brain. In effect, the metastasis of cancer cells to the brain may

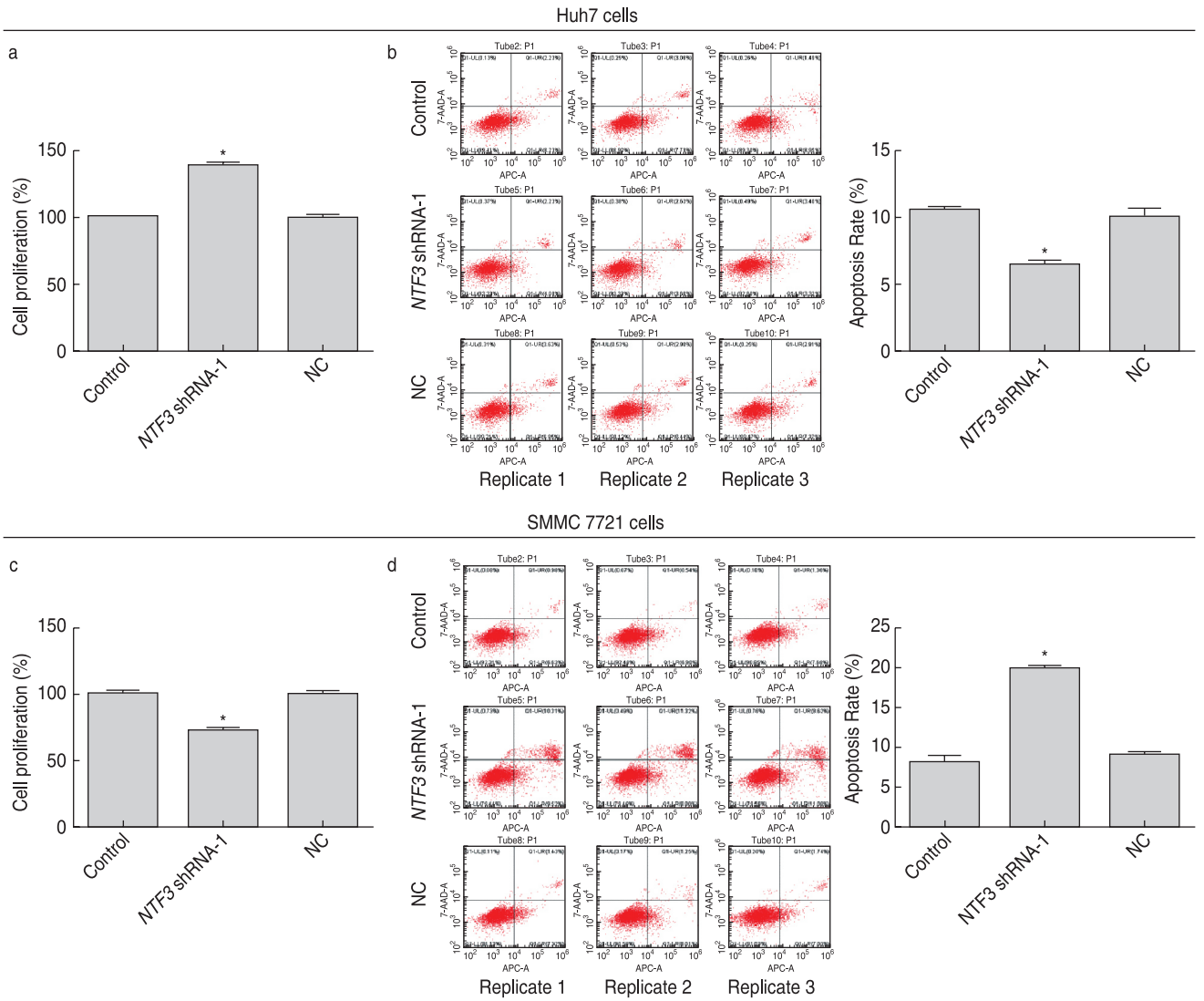

Fig. 3 *NTF3* inhibits proliferation and induce apoptosis of HCC cells. (a) Silencing of *NTF3* induces proliferation of Huh7 cells; (b) Silencing of *NTF3* hinders apoptosis in Huh7 cells; (c) Overexpression of NTF3 inhibits the proliferation of SMMC-7721 cells; (d) Overexpression of *NTF3* induces apoptosis in SMMC-7721 cells

induce the expression of *NTF3* in the brain, which could explain the increased expression of *NTF3* in the metastatic breast cancer cells in the brain. Our finding is the first to systematically demonstrate the downregulation of *NTF3* in HCC and its correlation with clinical characteristics. Our results imply that *NTF3* could play a significant role in HCC.

Previous studies have suggested that *NTF3* plays a functional role in the regulation of various cellular processes [19, 27–30]. However, the role of *NTF3* in HCC is still unclear. To uncover the function of *NTF3* in HCC, we silenced *NTF3* in Huh7 cells and found that it caused an increase in cell proliferation while inhibiting apoptosis. In addition, the overexpression of *NTF3* in SMMC-7721 cells was accompanied by decreased cell proliferation and increased apoptosis. Our results were contrary to those indicating that silencing of optineurin, which downregulates *NTF3* expression, increases apoptosis of RGC-5 cells [29] and that conditional knockdown of *NTF3* promotes neuronal apoptosis [30]. Similar results were reported for vascular smooth muscle cell proliferation [28]. Our results indicate that, despite its negative effect on apoptosis of various cells, *NTF3* induces the apoptotic cell death of HCC cells. Thus, we stipulated that *NTF3* could be used to kill cancer cells as a novel therapy.

Cell migration and invasion are critical processes involved in diverse physiological events as well as in the physiopathology of many disorders such as cancer [31–35]. Here, overexpression of *NTF3* inhibited the migration and invasion of HCC cells while contrary results were

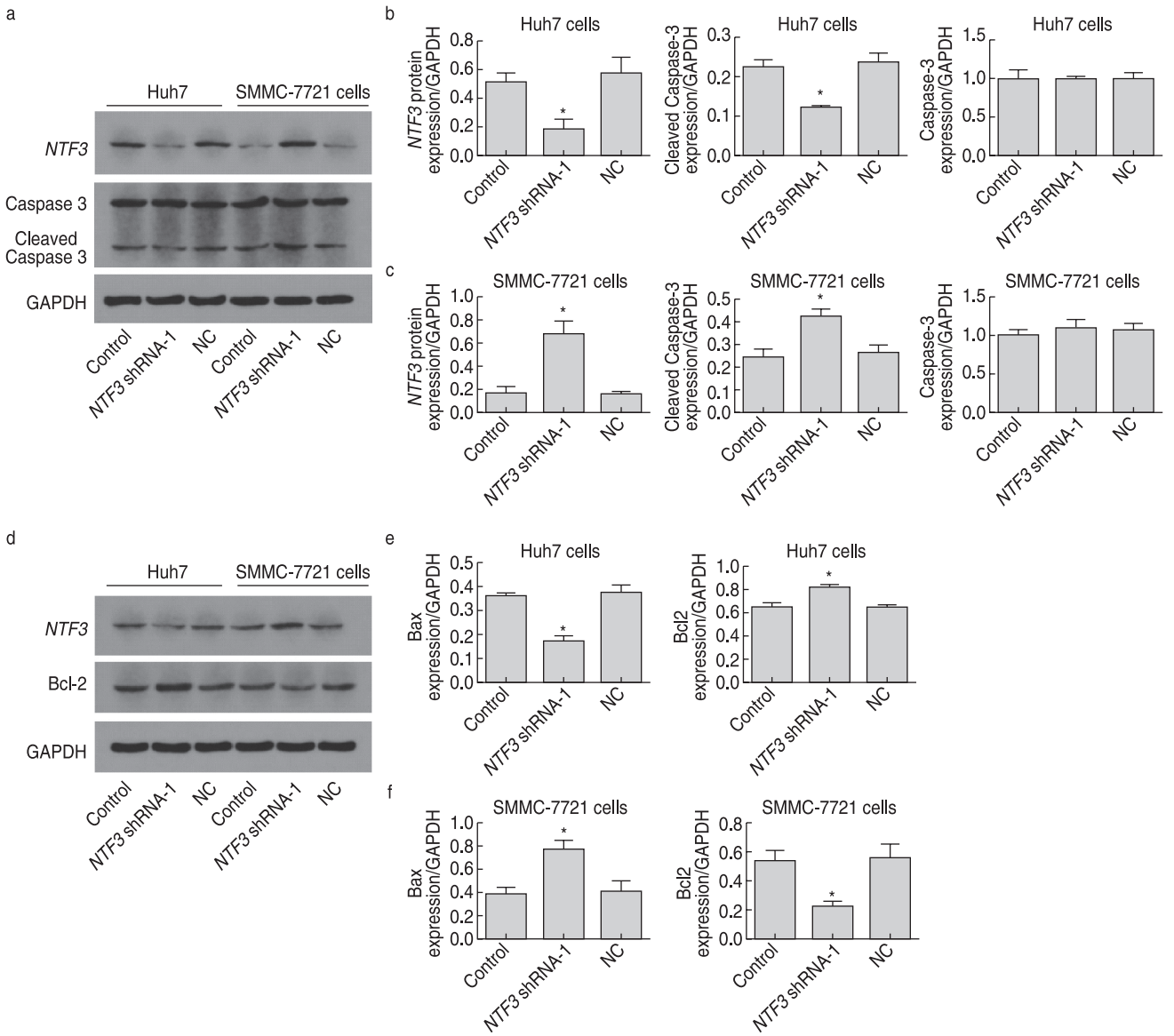

Fig. 4 *NTF3* regulates the expression of apoptosis markers in HCC cells. Western blot analysis of apoptosis markers was performed in Huh7 cells with silenced expression of *NTF3* and in SMMC-7721 cells

observed after *NTF3* silencing. Previous studies have indicated that SRY physically interacts with the *NTF3* promoter to synchronize cell migration in the testes during male sex determination [36]; however, the effect of *NTF3* on cell invasion has not been previously reported. Our study is the first to demonstrate that *NTF3* inhibits the migration and invasion of HCC cells. These results indicate that *NTF3* might inhibit the metastasis of HCC cells.

Conclusion

In this study, we examined the value of *NTF3* in HCC and demonstrated that (1) *NTF3* expression is decreased in HCC tissues and cells; (2) Decreased expression of *NTF3* is associated with a shorter survival time in HCC patients; (3) *NTF3* hinders proliferation, migration, invasion, and induces apoptosis of HCC cells. Owing to these results, we anticipate that *NTF3* might be a novel therapeutic target for HCC. However, further investigations are required for validating the effects of *NTF3* on the clinical course of HCC and on patient response to radiotherapy or chemotherapy.

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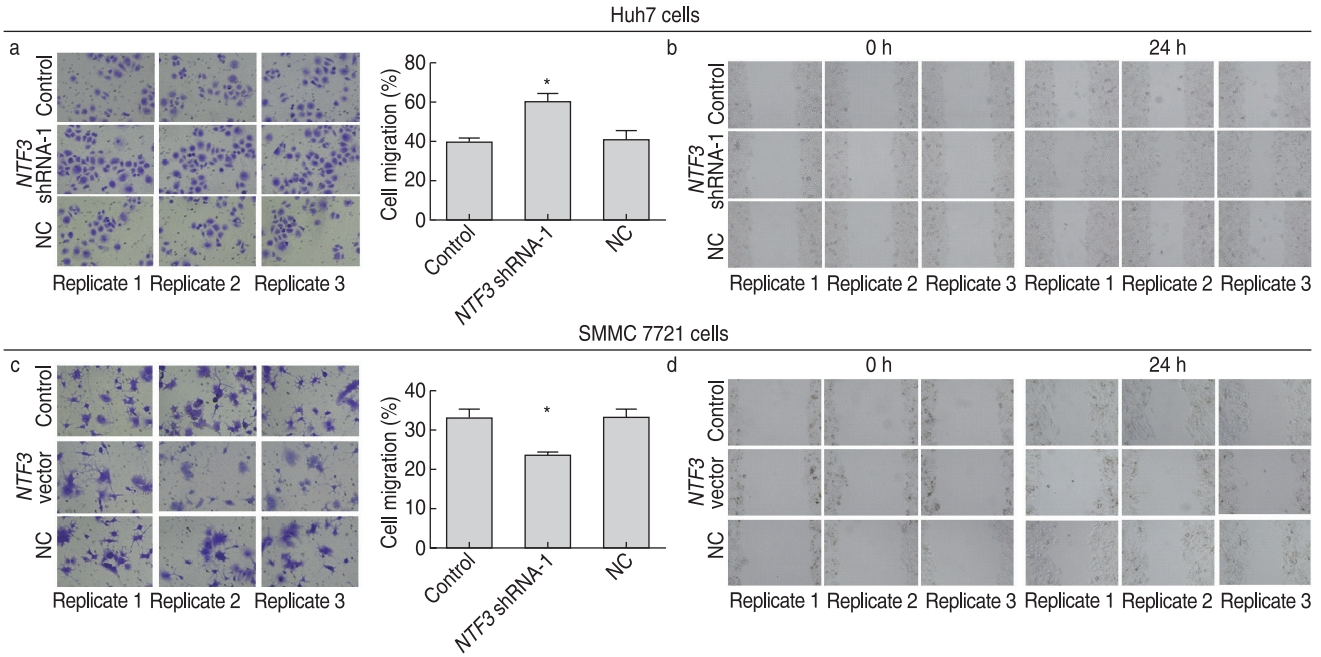

Fig. 5 *NTF3* inhibits invasion and migration of HCC cells. (a) Silencing of *NTF3* induces the invasion of Huh7 cells; (b) Silencing of *NTF3* induces the migration of Huh7 cells; (c) Overexpression of *NTF3* inhibits the invasion of SMMC-7721 cells; (d) Overexpression of *NTF3* inhibits the migration of SMMC-7721 cells

of Qingdao University, for advice on experimental design.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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ORIGINAL ARTICLE

Link between miR-19b and the mTOR signaling pathway in cancer prognosis*

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Abstract

Objective Previous studies have reported differing conclusions regarding the prognostic value of miR-19b in cancers. Moreover, miR-19b may affect tumor growth by different pathways, mainly targeting PTEN-PI3K-AKT, which activates the downstream mTOR pathway. Therefore, we performed data mining to explore the possible correlation between miR-19b and mTOR in cancer prognosis.

Methods We conducted online search and collected a total of 943 articles. According to different authors cross check and our study including/excluding criteria we at end retained 21 articles with 25 studies in this meta-analysis. Then TCGA data containing miR-19b level with cancer progression were obtained using OncomiR. Furthermore, Trial Sequential Analysis (TSA) was performed to determine whether the results of our meta-analysis could be used in clinical applications. After that, articles regarding the mechanism of miR-19b in various cancers were analyzed and KEGG pathway database was used to find the main regulatory function of miR-19b in human cancers.

Results Overall hazard ratio (HR) results showed that higher levels of miR-19b expression were correlated with shorter overall survival time [HR = 1.54, 95% confidence interval (CI) = 1.20-1.98] by promoting distant metastasis, but had no correlation with disease-free survival (DFS)/progression-free survival (PFS; HR = 0.61, 95% CI = 0.31–1.19). Data from The Cancer Genome Atlas also revealed the role of miR-19b in tumorigenesis. According to trial sequential analysis results, more evidence is required to confirm that miR-19b is not correlated with DFS/PFS. Exploration of the mechanism revealed a possible link between miR-19b and the mTOR pathway.

Conclusion miR-19b may have a pro-carcinogenic role through the mTOR pathway and thus, it is likely to be a therapeutic target for cancers.

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Key words: microRNA; *miR-19b*; prognosis; mechanism; mTOR; cancers

MicroRNAs (miRNAs) are a series of small endogenous single-stranded non-protein-coding RNA molecules with a length of 19–21 nucleotides [1]. They can regulate the expression of their target genes by binding to the 3'-untranslated region and affecting their translation or degradation [2–4]. The dysregulation of these genes plays significant roles in some pathways related to cancer processes, such as the cell cycle, adhesion, and motility [5]. Meanwhile, it is estimated that 60% of human genes are under the regulation of miRNAs [6], indicating that miRNAs might have certain roles in cancer progression [7–9]. Many recent studies have reported that miRNAs can be classified as either oncomiRs or tumor-suppressive miRNAs [3, 10–11]. Their abnormal levels have been associated with different aspects of cancer, including prognosis and clinicopathological features [12].

miR-19b is located on chromosome 13q31.3 and is recognized as the principal element of the *miR-17-92* cluster, which contains *miR-17*, *miR-18a*, *miR-19a*, *miR-19b*, *miR-20a*, and *miR-92* [13–15]. Recently, increasing evidence has demonstrated that *miR-19b* may be a prognostic biomarker in various human cancers, due to its close relationship with cancer prognosis [16–19]. Moreover, many cancer types, either with high or low mortality rates, have been found to be affected by *miR-19b*. These include astrocytic gliomas [20], nasopharyngeal

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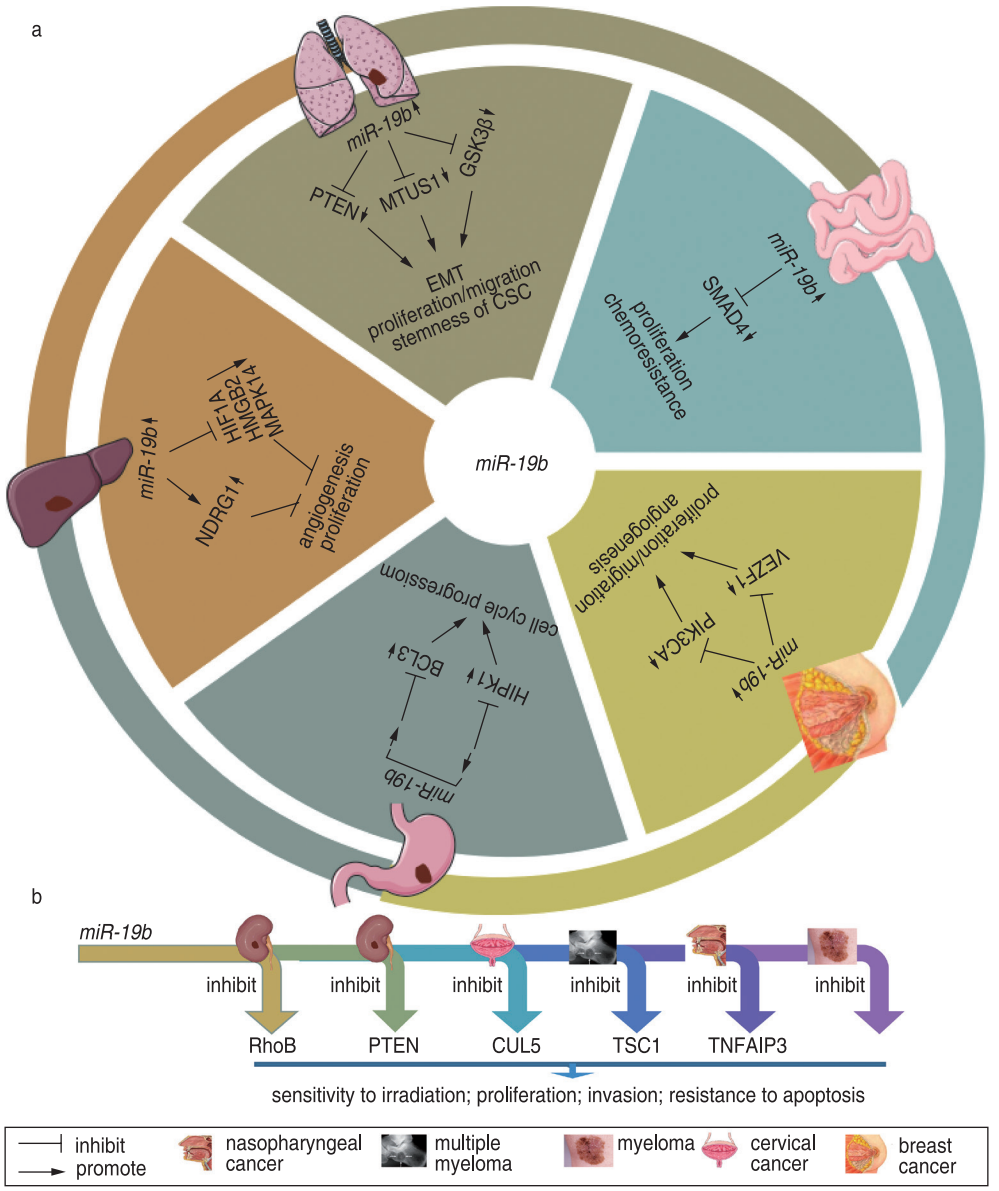

Fig. 1 Correlation between *miR-19b* and prognosis of cancer patients: (a) *miR-19b* roles in cancers with a high mortality rate; (b) *miR-19b* roles in cancers with a low mortality rate.

carcinoma [21], breast cancer [22–26], gastric cancer [27–28], lung cancer [29–31], liver cancer [32], colon cancer [17], renal cancer [33–35], cervical carcinoma [36], ovarian cancer [37], multiple myeloma [38] and melanoma [39]. These studies have confirmed the widespread roles of *miR-19b* in both high- and low-mortality-rate cancer types (Fig. 1). Fluctuating levels of *miR-19b* expression may affect tumor growth through different signaling pathways, but the prognostic role of *miR-19b* in different cancer types remains controversial. In addition, many studies have reported that *miR-19b* targets the PTEN-PI3K-AKT signaling pathway [22, 24–25, 33–34, 36–37, 40–42]. As a key kinase downstream of PI3K-AKT, mTOR can regulate tumor cell proliferation, growth, survival, and angiogenesis [43–44]. Therefore, we speculated that *miR-19b* may play a major role in cancers through the mTOR signaling pathway.

The majority of previously published meta-analyses have evaluated the diagnostic or prognostic value of miRNAs in cancers, but have not evaluated the association of miRNAs with specific pathways. Therefore, we performed this study to first assess the prognostic roles of *miR-19b* in human cancers and further explore the possible link between *miR-19b* and the mTOR signaling pathway based on this meta-analysis. These results may

provide new routes for the prevention and treatment of cancers.

Materials and methods

Our systematic review and meta-analysis was performed according to the recommendations of the PRISMA statement [45].

Literature search strategy

We comprehensively searched literature published up to November 25, 2019 using PubMed, Embase, Web of Science, and Cochrane Library databases. The search terms, [("*miR-19b*" or "microRNA-19b" or "*miR19b*") AND ("cancer" or "carcinoma" or "tumor" or "adenocarcinoma" or "neoplasm" or "neoplasia" or "malignancy" or "malignant") AND ("prognostic" or "prognosis" or "survival" or "outcome" or "recurrence" or "relapse" or "clinical features" or "clinicopathological parameters")] were used to identify the relevant studies.

Inclusion and exclusion criteria

Only those publications that met the following criteria were selected: (1) the relationship between *miR-19b* expression and patient prognosis was analyzed; (2) patients were separated into high/low groups based on *miR-19b* levels; and (3) sufficient data were provided to evaluate the prognostic role of *miR-19b*. The exclusion criteria were: (1) reviews, letters, case reports, animal trials, and expert opinions; and (2) studies without useful information.

Data extraction and quality assessment

Fundamental information from the included articles was carefully extracted by two authors. If the study only provided Kaplan-Meier curves, hazard ratios (HRs) and 95% confidence intervals (CIs) were manually calculated using Engauge Digitizer version 4.1 (https://zenodo.org/record/3941227). We preferably selected multivariate data when the article provided both uni- and multivariate results. The Newcastle-Ottawa Assessment Scale (NOS) was applied to evaluate the quality of the included publications, with a score equal to or greater than 6 indicating high quality.

Extraction and analysis of the Cancer Genome Atlas datasets

We used OncomiR to assess The Cancer Genome Atlas (TCGA) datasets relating *miR-19b* expression with cancer development. *miR-19b* expression data were available for 30 cancer types, including 9497 cases. Log2 mean expression values were used to compare *miR-19b* levels in normal and tumor tissues. Significance in tumor development was determined using a paired Student's *t*-test to compare *miR-19b* expression levels between normal and tumor tissues. Analysis of variance was performed to compare *miR-19b* expression levels between different cohorts for each clinical parameter.

Statistical analysis

Analyses were performed using Stata SE12.0 (STATA Corp, USA). Odds ratios (ORs) and 95% CIs were applied to analyze the relationship between *miR-19b* expression and tumor characteristics. The pooled HR and 95% CI were used to evaluate the prognostic value of *miR-19b*. HRs greater than 1 indicated that *miR-19b* was a factor leading to worse prognosis. Meanwhile, the Q test and I^2 statistics were assessed to evaluate the heterogeneity between included publications. $P < 0.05$ or $I^2 \geq 50\%$ indicated significant heterogeneity, in which case, we selected the random-effects model. Otherwise, we proceeded to the fixed-effects model. Additionally, subgroup and sensitivity analyses were performed to identify the source of heterogeneity, and publication bias was assessed by using a funnel plot and Begg's test.

Trial sequential analysis

Trial sequential analysis (TSA) can be used to avoid the risk of errors related to a small sample size. It can minimize the false positive/negative results caused by random errors. As shown in Fig. 2, curve A only crosses the traditional threshold (Z = 1.96), indicating that a false positive result may be obtained. However, more trials are needed to confirm this. Curve B crosses both the traditional and the TSA threshold, which indicates that a true positive result was obtained and no more trials are needed. Curve C crosses neither of the thresholds [Z = 1.96, TSA threshold and a priori information size (APIS)] and therefore, more trials are required to confirm the negative result. Curve D only exceeds the APIS, which indicates that there is no statistical difference and no more trials are needed to confirm the result. In this meta-analysis, TSA was performed to evaluate the reliability of

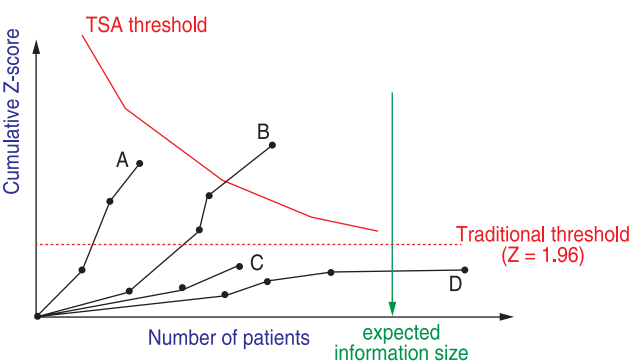

Fig. 2 Diagram of trial sequential analysis

our results, using the criteria of relative risk reduction = 15%, alpha = 5%, and statistical test power = 80%.

Target signaling pathway of *miR-19b*

We first generated an exhaustive collection of articles related to the mechanism of *miR-19b* in cancers. A series of information including the expression level of *miR-19b*, its target genes, its signaling pathways, and its role in cancer progression were extracted. The Kyoto Encyclopedia of Genes and Genomes (KEGG) database was then used to analyze the target mRNAs of *miR-19b* and their different signaling pathways, as reported in the literature. All of these processes were independently performed by two authors. Meanwhile, web-based tools, such as miRDB, miRTarBase, and TargetScan (http://mirdb.org/) were used to determine the effect of *miR-19b* on its target genes. Gene Ontology (GO) and KEGG pathway analyses of these genes were performed using R3.5.3 software, to verify the conclusions of the articles.

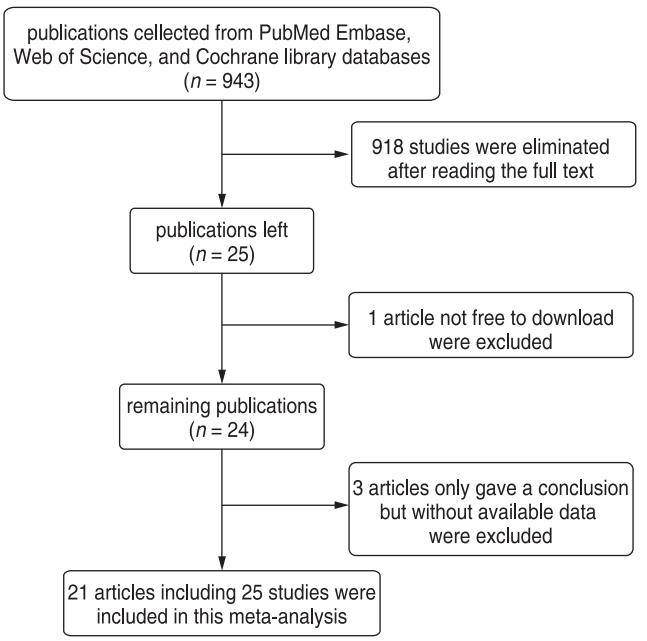

Fig. 3 Article search workflow and information extraction process

Table 1 HRs and 95% CIs of all selected studies

Outcome subgroup	First author, Year	Country	Cancer type	HR (95% CI)	*P* Value
OS (tissue)	Hung, 2015	China	HCC	0.318 (0.120–0.846)	0.022
	Wu, 2014	China	NSCLC	3.466 (1.389–8.650)	0.008
	Huang, 2016	China	NPC	2.967 (1.008–8.772)	< 0.05
	Li, 2018	China	BC	2.560 (1.130–5.796)	0.024
	Jiang, 2017	China	CC	2.23 (1.42–3.58)	0.008
	Wang, 2016	China	GC	0.62 (0.26–0.94)	0.002
	Marcela, 2016	Brazil	BL	0.54 (0.05–5.74)	0.207
	Xu, 2013	China	ESCC	1.77 (0.75–4.21)	0.764
	Zhao, 2017	China	BC	1.95 (1.13–3.37)	0.0092
	Huang, 2017	China	CRC	1.17 (0.21–6.53)	< 0.001
	Shao, 2018	China	GC	1.017 (0.981–1.054)	0.356
	Yu, 2012	China	CC	1.52 (1.09–2.11)	0.367
DFS (tissue)	Hung, 2015	China	HCC	0.455 (0.245–0.845)	0.013
	Jiang, 2017	China	CC	2.73 (1.76–3.89)	0.016
	Wang, 2016	China	GC	0.48 (0.21–0.87)	0.012
	Silvia, 2017	Spain	CRC	0.25 (0.08–0.78)	0.017
	Silvia, 2017	Spain	CRC	0.37 (0.14–0.98)	0.041
OS (Serum/Plasma)	Peng, 2018	China	GC	1.224 (0.856–1.751)	0.268
	Wu, 2014	China	NSCLC	1.800 (1.008–3.216)	0.047
DFS/PFS (Serum/Plasma)	Alfons, 2015	Spain	MM	0.10 (0.01–0.94)	< 0.0001
	Peng, 2018	China	GC	1.28 (0.947–1.729)	0.108
OS (BM)	Zhang, 2018	China	Non-M3 AML	1.68 (0.72–2.82)	0.118
	Zhang, 2018	China	CN AML	2.50 (0.98–5.25)	0.064
PR	Zhang, 2018	China	Whole AML	2.11 (1.62–3.67)	0.047
DFS (Urine)	Stuopelytė, 2016	Lithuania	PCa	0.45 (0.02–0.98)	0.014

Note: HCC: hepatocellular carcinoma; NSCLC: non-small cell lung cancer; NPC: nasopharyngeal carcinoma; BC: breast cancer; CC: colon cancer; GC: gastric cancer; BL: burkitt lymphoma; ESCC: esophageal squamous cell carcinoma; CRC: colorectal cancer; MM: multiple myeloma; AML: acute myeloid leukemia; CN AML: cytogenetically normal AML; PCa: prostate cancer.

Table 2 Pooled ORs for the relationship between *miR-19b* expression levels and clinicopathological features

Clinicopathological features	Studies	Heterogeneity			Model
		ORs (95% CIs)	I^2 (%)	*P*	
Tumor size (≤ 3cm *vs* > 3cm)	7	0.88 (0.46–1.69)	54.9	0.038	Random-effects
Tumor stage (I/II *vs* III/IV)	10	0.74 (0.30–1.81)	86.7	0.000	Random-effects
Vascular invasion (yes *vs* no)	4	0.94 (0.59–1.49)	44.9	0.142	Fixed-effects
LNM (yes *vs* no)	7	1.09 (0.39–3.01)	68.8	0.004	Random-effects
Tumor differentiation (W + M *vs* P)	9	1.00 (0.51–1.95)	66.2	0.003	Random-effects
DM (yes *vs* no)	3	3.43 (1.32–8.90)	56.1	0.102	Random-effects

Results

Study characteristics

Based on the aforementioned inclusion criteria, 943 articles were retrieved from PubMed, Embase, Web of Science, and Cochrane Library databases. After reading the entire text of these articles, 918 articles were eliminated due to the lack of useful information. Among the remaining 25 articles, one was not free to download and three others only gave a conclusion, without available data. Finally, 21 articles encompassing 25 studies were included in this meta-analysis (Fig. 3). A total of 2273 patients with 13 cancer types were distributed among the 21 articles. The fundamental information from these articles is summarized in Supplementary Table 1. All publications had NOS scores between 6 and 9, with an average score of 7 (Table 2). The HRs and 95% CIs of the articles are shown in Table 1.

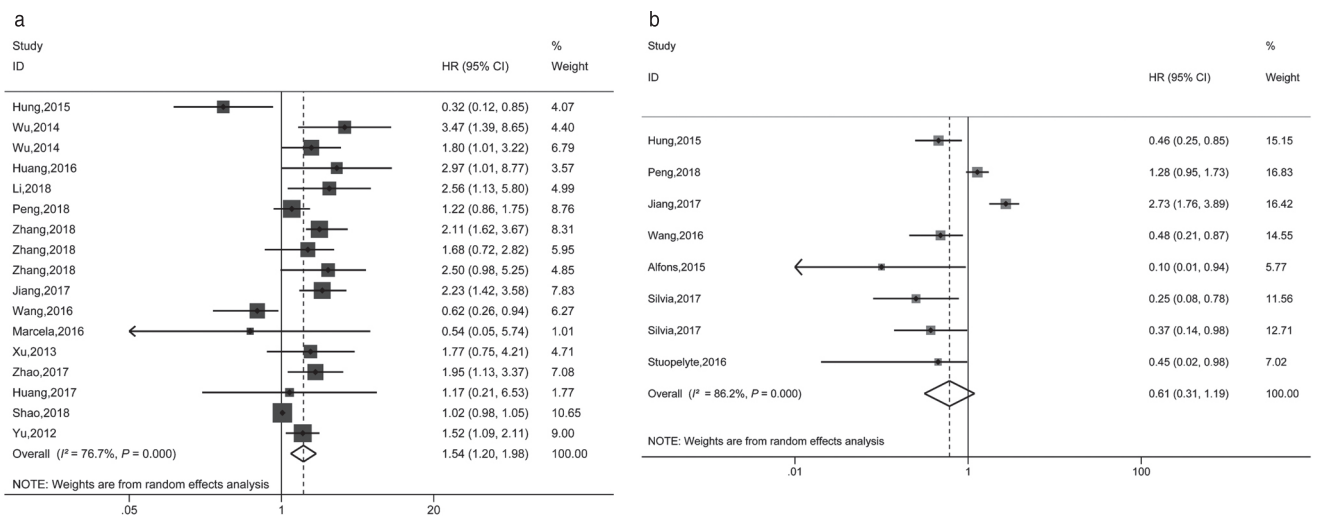

Fig. 4 Forest plots of the relationship between *miR-19b* expression levels and cancer patient prognosis: (a) OS; (b) DFS/PFS

Table 3 Relationship between *miR-19b* expression and cancer progression

Cancer type	Upregulated in	*P* Value	Clinical status	*P* Value	TCGA dataset
Bladder urothelial carcinoma	Tumor	< 0.0001	Pathologic M Status/Clinical T Status	0.0443/0.0265	TCGA-BLCA
Colon adenocarcinoma	Tumor	< 0.0001	Pathologic Stage	0.039	TCGA-COAD
Esophageal carcinoma	Tumor	0.00525	-	-	TCGA-ESCA
Kidney chromophobe	Normal	< 0.0001	Pathologic T Status	0.00184	TCGA-KICH
Liver hepatocellular carcinoma	Normal	0.00763	Pathologic N Status	0.0161	TCGA-LIHC
Lung adenocarcinoma	Tumor	0.00256	Pathologic T Status	0.0144	TCGA-LUAD
Lung squamous cell carcinoma	Tumor	0.000521	-	-	TCGA-LUSC
Prostate adenocarcinoma	Tumor	< 0.0001	-	-	TCGA-PRAD
Rectal adenocarcinoma	Tumor	0.0036	-	-	TCGA-READ
Stomach adenocarcinoma	Tumor	< 0.0001	-	-	TCGA-STAD
Thyroid carcinoma	Normal	< 0.0001	Pathologic Stage	0.000309	TCGA-THCA
Uterine corpus endometrial carcinoma	Tumor	< 0.0001	-	-	TCGA-UCEC

Correlation between *miR-19b* level and OS

A total of 17 studies were used to assess the correlation between *miR-19b* and overall survival (OS). Due to significant heterogeneity (I^2 = 76.7%, P < 0.05), the random-effects model was applied. Higher levels of *miR-19b* expression were found to be associated with shorter OS time (HR = 1.54, 95% CI = 1.20–1.98; Fig. 4a). Subgroup analysis (Fig. 5; Supplementary Table 3) further showed that *miR-19b* overexpression in tissues and bone marrow (BM) was correlated with poor OS in Asian populations (HR: 1.56, 95% CI: 1.21–2.01) and in groups with a sample size greater than 100 (HR = 1.86, 95% CI = 1.47–2.34). When cancers were classified as solid or non-solid tumors, we observed that *miR-19b* was more significantly associated with shorter OS time in non-solid (HR = 2.06, 95% CI = 1.49–2.84) than solid tumors (HR = 1.44, 95% CI = 1.10–1.88).

Correlation between *miR-19b* level and DFS/PFS

A total of 8 studies discussed the correlation between *miR-19b* levels and disease-free/progression-free survival (DFS/PFS). In line with the above analyses on the correlation between *miR-19b* and OS, we used a random-effects model (I^2 = 82.6%, P < 0.05) to explore its relationship with DFS/PFS. There was no correlation found between *miR-19b* levels and DFS/PFS (HR = 0.61, 95% CI = 0.31–1.19, Fig. 4b). Since *miR-19b* level of had no effect on cancer patient DFS/PFS, we did not perform further subgroup analyses.

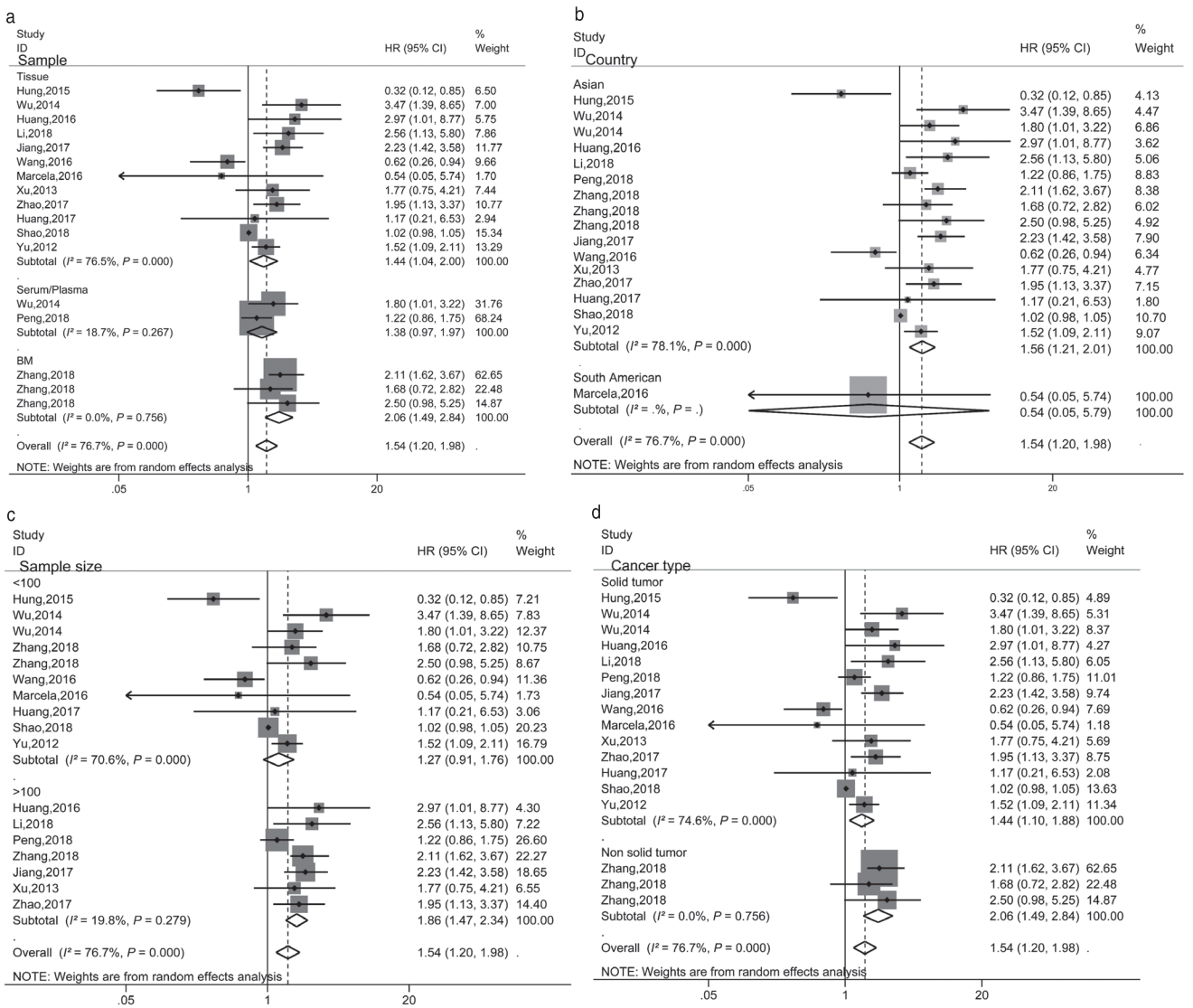

Fig. 5 Subgroup analysis of OS stratified by (a) detection sample; (b) study country; (c) sample size; and (d) cancer type

Correlation between *miR-19b* level and clinicopathological features

There were 13 studies that focused on the relationship between *miR-19b* and clinicopathological features, including tumor size, tumor stage, vascular invasion, lymph node metastasis, and distant metastasis (DM). As shown in Table 2, *miR-19b* level was only related to cancer DM (OR = 3.43, 95% CI = 1.32–8.90). Further subgroup analysis was not performed due to insignificant heterogeneity.

Correlation between *miR-19b* level and tumor progression in TCGA dataset

Under the predetermined significance threshold of $P \leq 0.05$, *miR-19b* expression was significantly associated with tumorigenesis in 12 cancer types from the TCGA dataset. *miR-19b* was significantly up-regulated in nine of these cancer types, but down-regulated in three others. In addition, a correlation between *miR-19b* level and clinicopathological status was also observed in some cancers. The relationship between *miR-19b* expression and cancer progression is presented in Table 3.

Sensitivity analysis and publication bias

Sensitivity analysis was carried out to identify which articles impacted heterogeneity. These results indicated that pooled HRs would not be greatly affected by excluding any study, which indicated that the above analyses were reliable and credible (Fig. 6a and 6b). We next applied funnel plots and Begg's test to estimate the publication bias of the included studies. The funnel plot, displayed in Fig. 6, showed *P* values of 0.902 for OS and 0.063 for DFS/PFS, indicating that there was no publication bias in this Meta-analysis.

Reliability and clinical applicability of results

We performed TSA to evaluate the reliability and clinical applicability of our results. From the results (Fig. 7), we can see that the cumulative Z-curve of OS was similar to curve B in Fig. 2, indicating that a true positive result was obtained. It stipulated that high expression of *miR-19b* was associated with poor OS. No more trials are needed to support this conclusion. In addition, the cumulative Z-curve of DFS/PFS looked like curve C in Fig. 2, which meant that more trials are required to prove

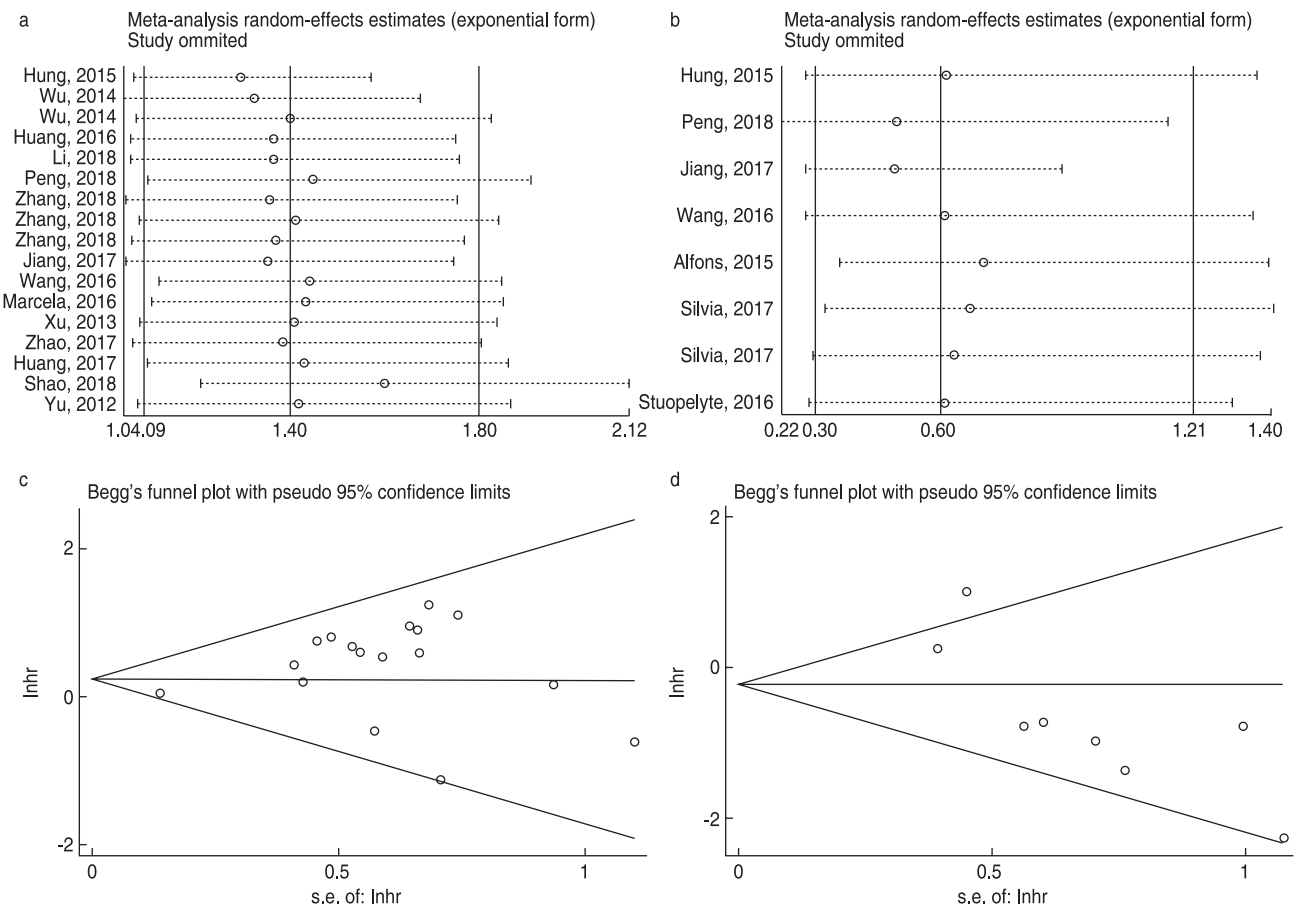

Fig. 6 Sensitivity and publication bias of studies: (a) sensitivity analyses for OS and (b) DFS/PFS and (c) funnel plot for OS (d) and DFS/PFS

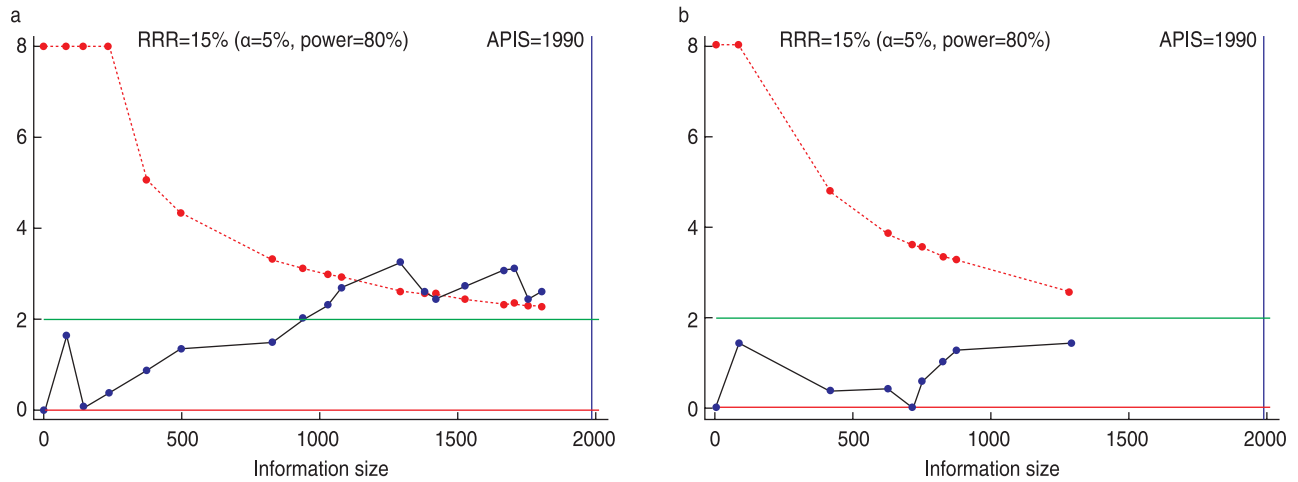

Fig. 7 TSA for cancer prognosis based on APIS: (a) OS; (b) DFS/PFS

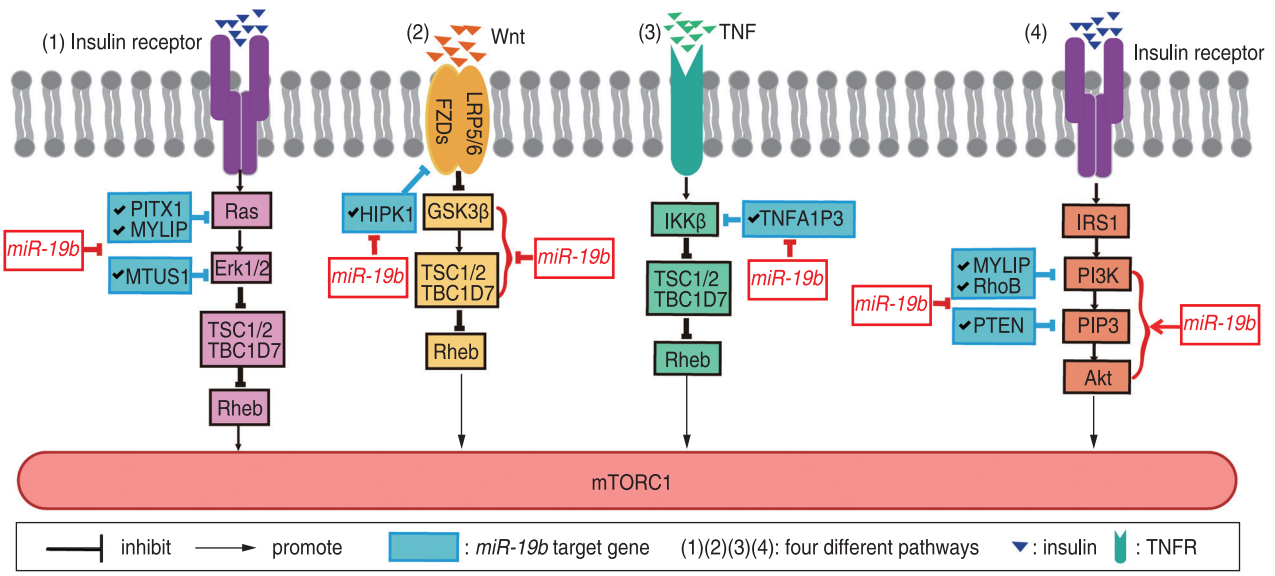

Fig. 8 Molecular mechanism showing the link between *miR-19b* and the mTOR signaling pathway in cancers

the conclusion that *miR-19b* was not correlated with DFS/PFS.

Connection between *miR-19b* and mTOR signaling pathway

To clarify the biological mechanism of the involvement of *miR-19b* in cancers, we reviewed published articles that focused on the target genes of *miR-19b* and its biological mechanism. The relevant information is listed in Supplementary Table 4. These data indicated that *miR-19b* was involved in various signaling pathways, but its involvement in the PTEN-PI3K-AKT signaling pathway was most notable. Some other studies also reported its involvement in the RAS and Wnt/β-catenin signaling pathways. Then, based on data from KEGG, we observed that mTOR was the intersection point of these different signaling pathways. These pathways could affect the mTOR signaling pathway through related molecules. A diagrammatic summary of the mechanism related to the correlation of *miR-19b* with the mTOR signaling pathway is presented in Fig. 8. Three additional databases miRDB, miRTarBase, and TargetScan, were then used to verify our findings. By intersecting the predicted target genes from these tools, 274 genes were selected and the functional annotations of these genes was performed by GO and KEGG analysis. As displayed in Fig. 9, these 274 genes mainly participated in the "mTOR signaling pathway", which verified our findings.

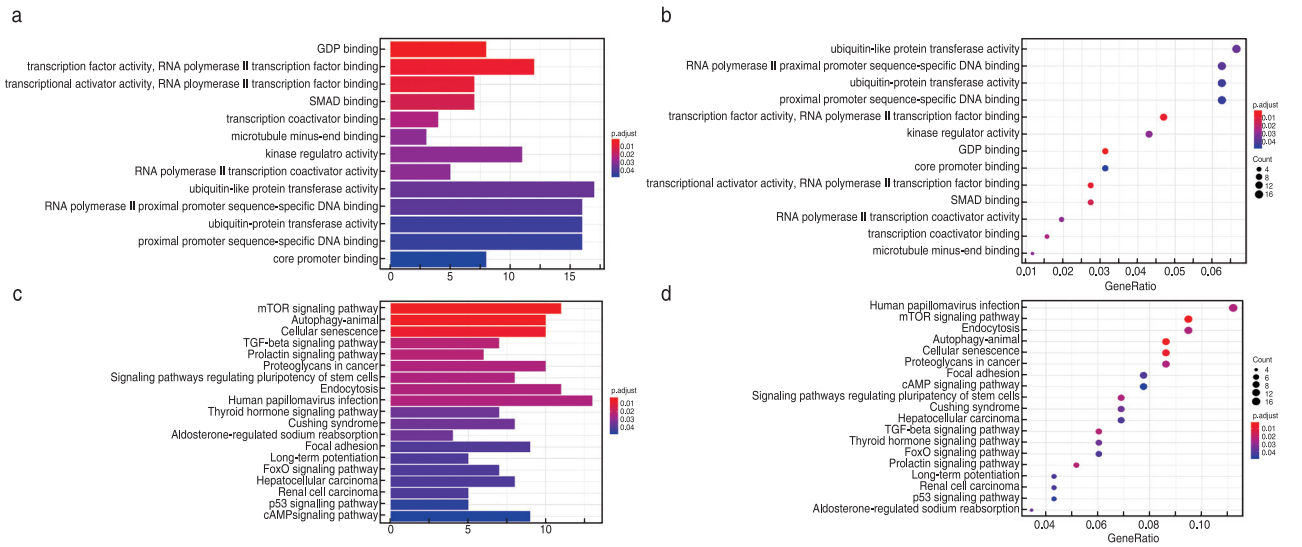

Fig. 9 Bioinformatics supporting the correlation between *miR-19b* and mTOR in cancers: (a) and (b) enrichment of the top 13 Gene Ontology terms, (c) and (d) Kyoto Encyclopedia of Genes and Genomes pathways.

Discussion

miR-19b, is a member of the *miR-17-92* cluster, which contains *miR-17*, *miR-18a*, *miR-19a*, *miR-19b*, *miR-20a*, and *miR-92*, and is located on chromosome 13q31.3. In 2004, Ota *et al* [46] discovered for the first time that the *miR-17-92* cluster is involved in the pathogenesis of B-cell lymphoma. A few years later, Mu *et al* [47] found that *miR-19b* was the principal element of the *miR-17–92* cluster. As a result, it has been widely speculated that *miR-19b* may serve as a biomarker in various types of cancers. Recently, an increasing number of studies have shown that *miR-19b* may serve as a predictor of unfavorable prognosis in cancer patients, because it may lead to the downregulation of tumor suppressor genes or the upregulation of tumor promoter genes. The overexpression of *miR-19b* is associated with poorer prognosis in patients with non-small cell lung cancer [48], nasopharyngeal carcinoma [21], breast cancer [22], ovarian cancer [37], acute myeloid leukemia [49], and colon cancer [17]. Julia *et al* [50] demonstrated that *miR-19b* regulates apoptosis-related activities in tumors through related genes. Wang *et al* [51] reported that *miR-19b* affects tumor growth and grade in gastric cancer. By contrast, some publications have reported that *miR-19b* plays inhibitory roles in tumor development, hence improving patient prognosis. This inhibitory role has been found mainly in hepatocellular carcinoma [32], gastric cancer [27], and multiple myeloma [52]. In other studies, no association between *miR-19b* and cancer prognosis has been found in Burkitt's lymphoma [53] and esophageal squamous cell carcinoma [54]. We can conclude that the prognostic roles of *miR-19b* in different cancers remain controversial. Therefore, we performed this meta-analysis to evaluate the prognostic and clinicopathological role of *miR-19b* in cancers.

The clinically relevant indicators of patient survival include OS, DFS, PFS, and recurrence-free survival (RFS). Due to the lack of relevant literature on RFS, in this meta-analysis, we assessed the relationship between *miR-19b* and OS and DFS/PFS. The results of pooled HRs suggested that high expression levels of *miR-19b* may result in shorter OS time (pooled HR = 1.54, 95% CI=1.20–1.98), whereas there was no influence on DFS/PFS (pooled HR = 0.61, 95% CI = 0.31–1.19). This difference observed in the prediction of OS and DFS/PFS by *miR-19b* may be due to differences in the measurement of these indicators in each study. These differences may result from bias in measuring PFS, difficulties during the end-point collection of DFS data, and the possible influence of other diseases. Nevertheless, OS represents the time from the observation period to the death of the patient.

Due to the large degree of heterogeneity among the included studies, four subgroup analyses were performed to further explore the role of *miR-19b* by sample type, country, sample size, and cancer type. The *miR-19b* level in tissues and BM had a clear impact on OS. This result indicated that *miR-19b* levels in tissues (for solid tumors) and BM (for non-solid tumors) were more meaningful than plasma or serum levels for predicting OS. However, increased *miR-19b* levels in BM were associated with a greater decrease in OS time (HR = 2.06, 95% CI = 1.49–2.84) when compared with increased *miR-19b* levels in tissues (HR = 1.44, 95% CI = 1.04–2.0). This conclusion requires further verified, since there were only three studies in the BM subgroup. When the sample size was

larger than 100, *miR-19b* could predict OS time (HR = 1.86, 95% CI = 1.47–2.34) and showed less heterogeneity, indicating that sample size could influence the accuracy of the conclusions of a study. When grouping patients by cancer type, we observed that *miR-19b* level was a potential biomarker of prognosis in both solid and non-solid tumors.

Based on the above results, we concluded that *miR-19b* has a wide application value in predicting OS time in patients with either solid or non-solid tumors, and high *miR-19b* expression levels lead to shorter OS time in cancer patients. After investigating the publications identified in online database searches, we analyzed TCGA data to further refine the conclusions drawn from the published articles. These data showed that the dysregulation of *miR-19b* was correlated with cancer clinical stage. This further indicated a major role of *miR-19b* in promoting cancers. Combined with the effect of *miR-19b* on DM (OR = 3.43, 95% CI = 1.32–8.90), we speculated that *miR-19b* may affect cancer prognosis by promoting DM. An increasing number of recent studies have suggested that circulating tumor cell (CTC) entry into the bloodstream to reach distant organs is a key step in the initiation of metastasis [55]. Vascular formation, immunosuppression, and epithelial-mesenchymal transition (EMT) of CTCs may be involved in DM. Interestingly, Li *et al* showed that high expression levels of *miR-19b* may promote EMT and thus, enhance the migration and invasion ability of lung cancer cells [29]. Wang *et al* even suggested that *miR-19b* may trigger EMT via exosomes secreted by clear cell renal cell carcinoma stem cells [35]. Mao *et al* [56] came to the same conclusion that *miR-19b* is largely involved in EMT via the *miR-19b*-PTEN-AKT signaling pathway.

Since high levels of *miR-19b* expression were correlated with poor prognosis in various types of cancers, it may be considered as a therapeutic target. A new therapeutic strategy may be possible by targeting the relevant pathway affected by *miR-19b* [57]. However, *miR-19b* plays different roles in cancers through different pathways. The relationship between these pathways and the mechanism by which they involve *miR-19b* in cancers remain unclear. Thus, it is critical to find a single pathway that connects these different pathways involved in the oncogene role of *miR-19b*.

Most of the reviewed articles reported that *miR-19b* overexpression activates the PTEN-PI3K-AKT pathway via the direct targeting of PTEN, RhoB, MYLIP, and CUL5 in pancreatic cancer [42], multiple myeloma [40], breast cancer [22, 24–25, 41], ovarian cancer [37], Wilms' tumor [34], clear cell renal cell carcinoma [33], and cervical carcinoma [36].

Ohira *et al*. found that PITX1, which acts as a negative regulator of the RAS pathway [58], is downregulated by *miR-19b* in melanoma [59]. Moreover, Gu *et al*. demonstrated that *miR-19b* overexpression plays a key role in downregulating MTUS1 [60]. MTUS1 has been shown to interfere with ERK2, which is a part of the RAS pathway [61]. These studies confirmed that *miR-19b* is correlated with tumor progression by targeting the RAS pathway.

Recently, Zhu *et al*. showed that *miR-19b* activates the Wnt/β-catenin pathway by directly targeting GSK3β in lung cancer [31]. Based on the study of Wu *et al*, it is well established that *miR-19b* modulates the Wnt-β-catenin signaling pathway by regulating HIPK1 [28]. Thus, *miR-19b* may affect cancer progression by activating the Wnt-β-catenin pathway.

Wang *et al*. showed that differential expression of *miR-19b* regulates the TSC1/mTOR signaling pathway in multiple myeloma [38]. Furthermore, *miR-19b* has been shown to suppress TNFAIP3 in nasopharyngeal carcinoma [21]. According to the KEGG pathway database, as a negative feedback of the NF-κB axis, the suppression of TNFAIP3 may activate NF-κB through IKKβ, which in turn may regulate mTOR. Interestingly, the study of Jiang *et al* first illustrated that *miR-19b* plays a key role in colon cancer progression via SMAD4 [17]. Voorneveld *et al*. then found that SMAD4 was associated with poor prognosis in colorectal cancer through the BMP pathway, rather than the TGF-β signaling pathway [62]. Moreover, Karner *et al*. confirmed that the BMP family activates the mTORC1 signaling pathway, which promotes the expression of protein anabolism genes [63]. We can conclude that *miR-19b* promotes colorectal cancer progression via the *miR-19b*-SMAD4-BMP-mTOR axis. In brief, the above analyses indicated that the pro-carcinogenic mechanism of *miR-19b* involved the activation of the mTOR signaling pathway.

In summary, these data indicated that *miR-19b* may act as an onco-miR through activation of the PTEN-PI3K-AKT, RAS, Wnt/β-catenin, and mTOR signaling pathways. Thus, it remains important to explore the possible connection between these pathways in the pro-carcinogenic role of *miR-19b*. As shown in Fig. 8, we observed that *miR-19b* played a major role in cancers through the mTOR signaling pathway, which is the intersection point of these different pathways. GO and KEGG analysis verified these findings. Recently, many studies have demonstrated that mTOR may be a therapeutic target for cancers [64–65]. Taken together, these findings indicated the potential to improve cancer treatment by regulating *miR-19b*-related mTOR signaling pathways.

In spite of the rigorous protocols adopted in each process of the analysis, the bias and limitations of this study cannot be ignored. Firstly, four studies were excluded after reviewing the available data. The absence of these articles may have impacted our analysis.

Secondly, HRs were manually extracted from the survival curves for some studies, which may have introduced some errors. Thirdly, we originally planned to also explore the anti-cancer effect of *miR-19b*, but we did not identify a sufficient number of articles for this analysis. Finally, more high-quality, large-sample-size publications are required to confirm our conclusions.

Despite the above limitations, this is the first study to link meta-analysis results with a specific mechanism. This meta-analysis is the first to identify the relationship between *miR-19b* and prognosis, showing that high expression levels of *miR-19b* lead to poor OS by promoting distant metastasis. However, DFS/PFS was not influenced by *miR-19b*. In summary, the results of our study indicate that *miR-19b* may have an oncomiR role through the mTOR signaling pathway.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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ORIGINAL ARTICLE

Correlation between clinicopathological parameters of lung adenocarcinoma and lymph node metastasis

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Abstract

Objective The aim of the study was to study the correlation between the clinicopathological parameters of lung adenocarcinoma and lymph node metastasis and identify the risk factors of lymph node metastasis.
Methods The data of 258 patients with postoperative lung adenocarcinoma (mainly based on their pathological data) were collected and analyzed, and their basic information was counted.
Results Maximum tumor diameter was found to be an independent risk factor for lymph node metastasis. The larger the maximum diameter of the tumor in patients with lung adenocarcinoma, the higher the likelihood of lymph node metastasis. Solid predominant adenocarcinoma with mucin production is as an independent risk factor for superior mediastinal and subcarinal lymph node metastasis. Primary adenocarcinomas in the lower lobe of the lung may have a higher rate of lymph node metastasis than those in the upper lobe.
Conclusion The known pathological subtypes of lung adenocarcinoma can be used for the prediction of lymph node metastasis in various regions and guide the dissection of lymph nodes that would improve patients' prognosis.

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Key words: lung adenocarcinoma; lymph node metastasis; pathological subtype; risk factors

Lung cancer is the most common malignancy, with the highest mortality rate, in China. High-resolution CT screening improves the detection of lung cancer, among which lung adenocarcinoma is the most common type [1]. In the 2015 WHO classification of lung tumors, lung adenocarcinoma was re-recognized [2], including adenocarcinoma *in situ* (AIS), minimally invasive adenocarcinoma (MIA), lepidic predominant adenocarcinoma (LPA), acinar predominant adenocarcinoma (ACI), and papillary predominant adenocarcinoma (PAP), solid predominant adenocarcinoma with mucin production (SPA), micropapillary predominant adenocarcinoma (MPA), and invasive mucinous adenocarcinoma (IMA). Subsequently, more studies have confirmed that the pathological subtype of adenocarcinoma is an independent predictor of disease-free survival, and lymph node metastasis is an important factor for long-term survival [3–4]. However, since there is no unified understanding of the adaptive scope and resection boundary in subpulmonary lobectomy and non-systematic lymph node dissection, more studies are needed to clarify the risk factors of the occurrence and development of tumors and of lymph node metastasis.

Therefore, it is necessary to analyze and summarize the rule of lymph node metastasis of lung adenocarcinoma and to clarify its risk factors to provide a reference for minimally invasive treatment of patients. This study aimed to investigate the correlation and risk factors between the clinicopathological parameters of adenocarcinoma and lymph node metastasis.

Materials and methods

Data of 258 patients with lung adenocarcinoma who met the inclusion criteria in the Department of Thoracic Surgery, Eastern District of the Affiliated Hospital of

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Table 1 Analysis of lymph node metastasis at the different lobular stations in 258 patients with lung adenocarcinoma

Number of lymph node stations	RUL (*n* = 89, %)	RML (*n* = 16, %)	RLL (*n* = 55, %)	LUL (*n* = 56, %)	LLL (*n* =42, %)
2	2 (2.2)	0	3 (5.5)	0	0
3	0	0	1 (1.8)	0	0
4	3 (3.3)	0	1 (1.8)	1 (1.8)	0
5	0	0	0	2 (3.6)	0
6	0	0	0	1 (1.8)	0
7	0	2 (12.5)	5 (9.1)	0	3 (7.1)
8	0	0	0	0	0
9	2 (2.2)	0	0	0	1 (2.4)
10	3 (3.3)	0	3 (5.5)	1 (1.8)	3 (7.1)
11	4 (4.5)	0	5 (9.1)	2 (3.6)	2 (4.8)
12	4 (4.5)	0	6 (10.9)	3 (5.4)	3 (7.1)
13	0	0	0	0	0
Total	18 (20.2)	2 (12.5)	24 (43.6)	10 (17.9)	12 (28.6)

Note: RUL, right upper lobe; RML, right middle lobe; RLL, right lower lobe; LUL, left upper lobe; LLL, left lower lobe

Qingdao University (Qindao, China) from November 2018 to April 2019, were retrospectively analyzed. The inclusion criteria were as follows: patients with complete imaging data within 30 days before surgery; patients with single lung adenocarcinoma who were evaluated as stage N0–N2 for feasible surgical resection according to the eighth TNM staging; those who underwent R0 resection of lung tumor, including sublobectomy, lobectomy, or pneumonectomy with systematic lymph node dissection; all postoperative specimens that showed paraffin sectioning pathology. The exclusion criteria were as follows: pathologically confirmed non-lung adenocarcinoma; preoperative chemoradiotherapy or targeted therapy; incomplete medical history; multiple pulmonary nodules. Preoperative routine examination included tumor marker tests, electrocardiogram (ECG), echocardiography, and lung function test of all patients. All patients underwent a systematic physical examination before surgery. Imaging examinations of the brain, chest, and abdomen, such as computed tomography or (CT), magnetic resonance imaging (MRI), emission computerized tomography (ECT), and positron emission tomography (PET) were performed on some patients to determine that lung cancer is confined to one side of the chest without distant metastasis.

During the operation, at least three lymph nodes stations were dissected for each case, and the lymph node partitioning method was adopted: Group 2–6 was the superior mediastinal lymph region; Group 8–9 was the inferior mediastinal lymph region; Group 10–13 was the intrapulmonary lymph node (N1); and subcarinal lymph node was partitioned separately. The pathological report included tumor size (maximum tumor diameter as the evaluation index), site, histopathological type, total number of lymph nodes dissected, and positive number.

SPSS 18.0 software was used for data collation and analysis. Results were analyzed by using Pearson χ^2 test or calibration χ^2 test and logistic regression analysis. A *P*-value of less than 0.05 was considered statistically significant.

Results

There were 258 patients, including 88 males and 170 females, with a mean age of (59.3 ± 9.2) years. There were 89 cases of right upper lobe (34.5%), 16 cases of right middle lobe (6.2%), 55 cases of right lower lobe (21.3%), 56 cases of left upper lobe (21.7%), and 42 cases of left lower lobe (16.3%). The details are shown in Table 1. Among the pathological subtypes, there were 52 (20.2%) LPA, 128 (49.6%) ACI, 13 (5%) PAP, one (4.7%) MPA, 12 (4.7%) SPA, and nine (3.5%) IMA cases. A total of 1449 groups of 3499 lymph nodes were dissected. The mean number of lymph nodes dissected in each patient was 13.56 in 5.62 groups; 66 (4.6%) were positive, and 130 (3.7%) lymph nodes were metastatic, as shown in Table 2.

In tumors located in the right upper lobe, which included 89 cases, the lymph node metastasis rate was 20.2% (18/89). The frequency of lymph node metastasis from high to low in turn to were intrapulmonary lymph node (11–13) 9%, hilus pulmonis 3.3%, right lower paratracheal lymph node (4R) 3.3%, superior paratracheal lymph nodes (2R) 2.2%, inferior mediastinal lymph node (8–9) 2.2%. Among the 55 patients whose primary tumor was located in the right lower lobe, the total lymph node metastasis rate was 43.6% (24/55). The stations (11–13), subcarinal lymph node, and hilar lymph node were more prone to metastasis, and their metastasis rates were 20%, 9.1%, and 5.5%, respectively. There were 56 patients with a primary tumor in the left upper lobe, and the total lymph node metastasis rate was 17.9% (10/56), which was similar to that in the right upper lobe. The station lung

Table 2 Single factor analysis of the risk factors for regional lymph node metastasis

Influence factor	Superior mediastinal region		Subcarinal region		Inferior mediastinal region		N1 region	
	Positive cases	*P*	Positive cases	*P*	Positive cases	*P*	Positive cases	*P*
Gender								
female	5	0.256	6	0.952	1	0.232	18	0.498
male	6		4		2		7	
Age (years)								
< 60	3	0.413	8	0.043	0	0.341	12	0.656
≥ 60	8		2		3		13	
Lung lobe								
RUL	3		0		2		5	
RML	0		2		0		0	
RLL	4	0.125	5	0.002	0	0.392	9	0.074
LUL	4		0		0		5	
LLL	0		3		1		6	
T								
Tis	0		0		0		0	
T1	3	0.002	5	0.140	1	0.460	14	0.006
T2	8		5		2		11	
T3	0		0		0		0	
Subtype								
LPA	0		1		0		1	
ACI	8		6		2		14	
PAP	0	0.007	0	0.046	0	0.445	1	0.000
MPA	0		0		0		1	
SPA	3		3		0		6	
IMA	0		0		1		1	

Note: RUL, right upper lobe; RML, right middle lobe; RLL, right lower lobe; LUL, left upper lobe; LLL, left lower lobe; LPA, lepidic predominant adenocarcinoma; ACI, acinar predominant adenocarcinoma; PAP, papillary predominant adenocarcinoma; MPA, micropapillary predominant adenocarcinoma; SPA, solid predominant adenocarcinoma with mucin production; IMA, invasive mucinous adenocarcinoma

lymph nodes with the highest lymph node metastasis rate were 8.9%, the main-pulmonary artery window lymph node (5) was 3.6%, and the lymph node region with the lowest metastasis rate was the inferior mediastinal lymph node (0%), which was similar to the right upper lobe. There were 42 patients with tumors in the left lower lobe, and the total metastasis rate was 28.6% (12/42). The most easily metastatic lymph node region was the N1 region 19.0% (8/42), followed by the subcarinal region 7.1% (3/42). There were 16 patients with a primary tumor in the right middle lobe, and only 12.5% (2/16) subcarinal lymph node metastasis was observed in this study.

In 258 patients, the N1 lymph node metastasis rate was 15.1% (39/258), N2 lymph node metastasis rate was 10.5% (27/258). Among them, the single N1 metastasis rate was 4.7% (12/258), only 30.8% of all N1 lymph nodes (12/39). The N2 division alone – hop lymph node metastasis, lymph node metastasis rate was 2.7% (7/258), while N1 and mediastinal lymph node metastasis occurred in 13 cases (5.0%). Among the primary pulmonary lobes, the N1 region had the highest metastasis rate, with the right lower lobe being the largest (25.5%). In the mediastinal lymphatic region, the subcarinal lymphatic region had the highest metastasis rate of 37.0% (10/27). In 89 patients with N2 lymphatic metastasis in the right upper lobe, the mediastinal lymphatic region above was dominant, accounting for 71.4% (5/7) of the N2 region. Only two patients had metastasis in the inferior mediastinal region. The same characteristics were observed in the left upper lobe in 56 cases as in the right upper lobe. Unlike the primary upper lobe pattern, the probability of non-regional lymph nodes in patients with primary lower lobe is higher than that with upper lobe. Subcarinal lymph nodes were the most easily metastasized N2 lymph nodes in the right lower lobe, accounting for 50% (5/10). Lymph node metastasis in the left lower lobe was similar to that in the right lower lobe, and subcarinal lymph nodes accounted for 75% (3/4) of the N2 lymph nodes.

Single-factor analysis, subcranial lymph node metastasis is more likely to happen in patients older than 60 years with a significant difference of $P = 0.043$ and patients with right lower lobe compared to the rest of the lung to subcarinal lymph node metastasis rate higher statistically significant ($P = 0.002$). Compared to other subtypes of adenocarcinoma, subcarinal and N1 lymph node metastasis rates were higher with SPA (subcarinal

Table 3 Multivariate logistic analysis of the risk factors for regional lymph node metastasis

Risk factors	Superior mediastinal region (*P*)	Subcarinal region (*P*)	Inferior mediastinal region (*P*)	N1 region (*P*)
≥ 60 years old	0.354	0.024	0.996	0.550
The maximum diameter	0.000	0.013	0.038	0.001
Lower lobe	0.721	0.025	0.760	0.049
SPA	0.021	0.015	0.999	0.002

Note: SPA, solid predominant adenocarcinoma with mucin production

area $P = 0.046$, N1 area $P = 0.000$). T2 in the superior mediastinal region and N1 region showed statistical differences compared to T1 (superior mediastinal region $P = 0.002$, N1 region $P = 0.006$; Table 2).

In multiple factors analysis, tumor diameter was found to be an independent risk factor for lymph node metastasis. SPA in the mediastinal area ($P = 0.021$), subcarinal area ($P = 0.015$), and N1 area ($P = 0.002$) can be used as independent risk factors for lymph node metastasis. Tumors in the lower lobe occurring in the subcarinal area and N1 area are independent risk factors for lymph node metastasis (Table 3).

Discussion

For patients with lung adenocarcinoma, an accurate N stage is better for guiding the treatment and evaluating the prognosis. There is still a debate on how the preoperative N stage can be assessed accurately, and most doctors do not routinely use mediastinoscopy [5]. Although some researchers have tried to predict mediastinal lymph node metastasis [6], most of these researchers focused on only a particular histological type. The predictive value of the new classification of lung adenocarcinoma for patient survival and recurrence rate has been extensively studied. However, there is no evidence of correlation between subtypes and lymph node metastasis. Zhang *et al*[7] found that the lymph node metastasis rate of adenocarcinoma dominated by MPA and SPA was significantly higher than that of other subtypes, and the pathological subtypes were also analyzed as independent predictors of N0 metastasis ($P = 0.008$). Qin *et al*[8] found that MPA and SPA were independent risk factors for the upregulation of the N stage in clinical stage Ia adenocarcinoma. Studies have found that selective lymphadenectomy can be considered for better-differentiated subtypes (such as AIS, MIA, and LPA) [9–11], which will have greater benefits. This study also found that SPA was a risk factor for regional lymph node metastasis. However, there was no significant difference in the number of micropapillary cases.

There are some prospective studies [12] that continue to think that systemic cleaning of lymph nodes should be performed to improve the postoperative survival rate of patients with lung cancer following surgical treatment. The reason is that there can be a more accurate N stage to guide postoperative treatment [13] and can reduce the postoperative recurrence and metastasis; therefore, lobectomy and systemic lymph node cleaning techniques are still suggested as a standard procedure. However, more and more studies show that some patients with early lung cancer do not benefit from systematic cleaning [14], increasing the difficulty of surgery, surgical trauma, and some postoperative complications. Some patients had preoperative biopsy pathology, but few had lymph node biopsy results. Pathologic puncture results are helpful in guiding the surgical approach. Based on the correlation between clinicopathological parameters of adenocarcinoma and the lymph node regions in this study, lymph node dissection or lymph node sampling can be specifically selected during the operation without affecting the N stage to reduce surgical trauma. Combined with intraoperative rapid freezing pathology [15], intraoperative selective cleaning or sampling is feasible for early differentiation typing, which can increase the benefit in patients. Therefore, it is suggested that puncture pathology or freeze pathology should be accurate for the pathological subtypes. If conditions permit, accurate molecular typing can better analyze the patient's condition and allow more favorable conditions for long-term survival.

Based on the regional study of lymph nodes conducted in this study, the relationship between different pulmonary lobes and different pathological subtypes for lymph node metastasis is different. Lobe-specific lymph node dissection (L-SLD) determines the range of lymph node dissection according to the location of the tumor in the lung [16–17]. It is primarily applicable to patients in clinical stages I–II, and particularly for patients with a diameter < 2 cm; further, only when the main lymph node drainage area is frozen and pathologically negative can the lymph nodes in the non-drainage area not be dissected. However, the existence of skip transfer makes L-SLD controversial, and prospective research [18] is underway. This study is a retrospective analysis, and the data can be used as reference for future studies.

This study has limitations. For instance, there are errors and losses in the collection of medical history data and thus the findings need to be further proved using a

prospective multicenter randomized controlled study. The number of cases included in this study is small, and the positive sample size is less than five in the subgroup analysis, which may produce false-negative results. The single-center study lacks the calibration of different reference indexes. Hence, the research conclusions should be extended carefully. The correlation between lymph node metastasis and lung adenocarcinoma typing remains to be further studied.

Based on this study, the following conclusions are drawn: primary adenocarcinomas located in different pulmonary lobes have different metastasis patterns corresponding to the lymph node regions, and the lower lobe of the subcarinal lymph node region has a higher metastasis rate, which is an important area for dissection. Patients with lung adenocarcinoma are more likely to have lymph node metastasis as the maximum tumor diameter (T stage) increases. Based on the risk factors identified in this study, it can be concluded that the greater the transition probability of lung adenocarcinoma to the regional lymph nodes, the greater the reference line; further mediastinal lymph node stage, the lower the risk factors for prediction of smaller patients, under the condition of no surgery taboo still suggest the complete resection of the tumor and systemic lymph node cleaning to help improve the postoperative survival rate.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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REVIEW ARTICLE

Mental health status of cancer caregivers, assessment tools, and psychological interventions*

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Abstract

Many studies pointed out that psychological pain is not limited to the cancer patients themselves, but their caregivers also experience different levels of psychological problems such as depression, anxiety, and stress. This article attempts to review the mental health status, assessment tools, and psychological interventions of the caregivers of cancer patients, and calls on social and medical workers to pay attention to the mental and physical health status of the caregivers of cancer patients.

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Key words: cancer patient caregivers; mental symptoms; evaluation tools; psychological intervention

Globally, cancer is the second leading cause of death, causing 8.8 million deaths in 2015, and nearly one-sixth of all deaths [1]. Providing care to patients with cancer can also be overwhelming, and caregivers are at risk for physical and psychological distress that will affect their physical and mental health, and affect their quality of life for a long time. A cancer caregiver is defined by most studies as "the person who spends the most time caring for the patient and does not get paid" [2–3]; it usually refers to the family members who have the primary responsibility for the patient, such as spouse, children, parents, siblings, etc.

Nowadays, the length of hospital stays has been shortened, and the increase in the shift to outpatient services has placed a heavy burden of responsibility on caregivers, many of whom rarely prepare for it. The rapid development in the field of cancer care has improved our ability to extend lifespan and improve survivability. In many cases, cancer has become a chronic disease rather than a sudden life-limiting disease. These trends have greatly increased the burden on caregivers and, consequently, their needs.

Many investigations and studies [4] show that the prevalence of mental illness in the primary caregivers of cancer patients is 20% to 30%, while that of patients with advanced cancer or relatives of palliative care is 30% to 50%. Other studies [5–6] pointed out that the incidence of psychological problems in cancer patients has reached 100%. In addition, studies in South Korea show that 17.7% of cancer caregivers have been suicidal, and 2.8% of cancer caregivers have attempted suicide [7]. Therefore, whether it is for the care of cancer patients or for the physical and mental health of cancer caregivers, this group deserves social attention.

Mental symptoms of cancer caregivers

Depression

Depression is a common mood disorder among the caregivers of cancer patients. Grunfeld's study [8] showed that 30%–50% of cancer caregivers (here referred to as family caregivers) had different emotional responses, of

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which depression was one of the most common emotional responses. In a study of the caregivers of patients with advanced gastric cancer [9], 62.57% of caregivers had depressive symptoms.

Anxiety

Anxiety is also one of the common emotions of cancer caregivers. A study by Park [10] showed a high degree of anxiety in the caregivers of cancer patients, and a study of primary caregivers for breast and prostate cancer by Grov [6] showed that both male and female caregivers had significantly higher levels of anxiety than the normal level. Liu's research [11] also showed that the major caregivers of cancer patients who were hospitalized for chemotherapy had severe anxiety.

Stress

As both the stress level and care requirements for cancer care are higher than for general disease care, research has shown that [12] many cancer caregivers provide care as a result of increased emotional stress. Half of the cancer caregivers report that they experience high levels of emotional stress, while a small number of non-cancer caregivers report that providing care can be very emotionally stressful and that the emotional response of cancer caregivers can have a significant impact on survivor outcomes, including the survivor's own emotional response.

Cancer caregiver mental health assessment tools

A 2016 report on cancer caregivers published in the United States mentioned that pain screening or assessment should be performed for caregivers and that the appropriate resources should be provided [12]. The tools commonly used in clinical work to assess the mental health of caregivers are as follows:

The Burden Scale for Family Caregivers (BSFC)

The Family Carer Burden Scale was developed by Gräsel [13] in 1993 and was originally developed in Germany. As a clinical tool that measures the self-reported burden of home caregivers of relatives. The scale has 28 entries and uses a 4-point scale from 0 (strongly disagree) to 3 (strongly agree). A higher score indicates a greater burden on the caregiver. It provides basic information about the negative effects of care and how care affects the health of caregivers. The advantage of BSFC is that it can be used both as a clinical tool (such as to assess care and identify areas of interest) and for research purposes (such as for observational studies or as a measure of the outcome of intervention studies). Cronbach's α of the scale is 0.91 [14], which has been translated into multiple languages. The Cronbach's α of the Turkish version of the family caregiver burden scale is 0.89 [15] and the Danish version of the Cronbach's α is 0.91 [16].

The Burden Scale for Family Caregivers-short version (BSFC-s) [17]

The BSFC-s is a short version developed based on the Family Carer Burden Scale. It uses a 4-point scale from 0 (strongly disagree) to 3 (strongly agree). Scores range from 0 to 30, with higher scores indicating a greater burden on caregivers. The Cronbach's α of this scale is 0.92 [18], and it has been widely used in many languages.

Caregiver Burden Scale (CBS) [19]

There are 22 items in the CBS, and a 5-point Likert scale is used. Divided into five dimensions (general strain, isolation, disappointment, emotional involvement, and environment), it covers important areas such as health, mental health, interpersonal relationships, physical burden, social support, economics, and family environment. The scale measures scores and total scores for each dimension, and this helps to understand which specific dimensions have the most impact on caregivers. The questionnaire can be filled-out by the main test subject or by the test subject. A higher score indicates a greater burden on the caregiver. Except for the environment dimension, the Cronbach's α of other dimensions is 0.70–0.87 [20], and the Cronbach's α of the Turkish version of CBS is 0.91 [21].

Caregiver Reaction Assessment (CRA)

The CRA was developed by Given *et al* [22] in 1992 and was originally used to assess the response of elderly caregivers toward long-term care. Divided into four negative dimensions, Impact on Health (IH), Impact on Schedule (IS), Impact on Finances (IF), Lack of Family Support (LFS), and one positive dimension, Caregiver's esteem (CE), for a total of 24 entries, using a 5-point Likert scale. The higher the negative dimension score, the heavier the stress load of the caregiver, and the higher the positive dimension score, the lighter the stress load. The scale is characterized by both positive and negative dimensions and considers the participants' evaluation of positive and negative experiences. Studies evaluating the caregivers of stroke patients have shown that the Cronbach's α of each subscale is 0.62 to 0.83 [23]. CRA is currently widely used to assess the burden of caregivers of patients with various chronic diseases such as cancer, stroke, and Alzheimer's disease [24]. Some domestic scholars have shown that the Cronbach's α is 0.612–0.732 [25] in the reliability and validity test of the Chinese version of the caregiver response assessment scale which has good reliability and validity. However, some scholars have pointed out that the reliability of the

dimension Impact on Finances (IF) in the Chinese version of CRA is not good enough [26] and needs further revision and testing.

Zarit Caregiver Burden Interview (ZBI) [27]

The ZBI consists of 22 items rated on a 5-point Likert scale that ranges from 0 (never) to 4 (nearly always) with the sum of scores ranging between 0–88, and higher scores indicate a greater burden. A score of 17 or more indicates a heavy burden. Dimensions reported include consequences of caregiving, patient's dependence, exhaustion and uncertainty, guilt or self-criticism, embarrassment/anger or frustration, psychological burden and emotional reactions, personal strain, and role strain. The ZBI's psychometric proprieties have been extensively examined in the caregivers of patients with dementia and demonstrate strong evidence for reliability and validity in that population. The ZBI has also been examined in the caregivers of patients with cancer and brain injury. The reported Cronbach's α for the ZBI in caregivers of patients with cancer and dementia ranged between 0.85 and 0.93. [28]

Caregiver Strain Index (CSI)

The CSI was developed by Robinson [29] and used to quickly identify families with potential caregiving concerns. It is a 13-question tool that measures the strain related to care provision. There is at least one item for each of the following major domains: Employment, Financial, Physical, Social, and Time. The scale's answer method is "Yes" (1 point) or "No" (0 point), with a total score of 0 to 13 points. The higher the score, the higher the stress of the caregiver. When the cumulative score is ≥ 7, it indicates that the stress level is higher [30]. The Cronbach's α of CSI is 0.86 [29], and the Chinese version of Cronbach's α is 0.828 [31]. As the CSI has only 13 items and the answer method is in the form of "Yes" or "No", it can quickly assess the stress of the participants. It is also widely used in clinical practice, but it also has certain shortcomings. For example, this tool is limited by lack of a corresponding subjective rating of caregiving impact. There is no breakdown of score regarding low, moderate or high caregiver strain. The carer's stress level is qualitatively evaluated.

Caregiver Burden Inventory (CBI)

The CBI is a tool developed by Novak *et al* [32] for assessing the burden of caregivers and was initially used for caregivers of patients with Alzheimer's disease. The 24-item multi-dimensional questionnaire measures caregiver burden with 5 subscales: (a) Time Dependence; (b) Developmental; (c) Physical Burden; (d) Social Burden; and (e) Emotional Burden. Scores for each item are evaluated using a 5-point Likert scale ranging from 0 (not at all disruptive) to 4 (very disruptive). The Cronbach's α of the Chinese version of CBI is 0.85, and the Cronbach's α of the physiological, emotional, social, time-dependent, and development-restricted dimensions are 0.83, 0.88, 0.82, 0.90, and 0.87, respectively [33]. Some scholars have also suggested that although the reliability and validity of the Chinese version of the CBI supports the use of the Chinese version of the CBI as a research tool to measure the burden of Chinese caregivers, further research is needed to distinguish the burden of developmental constraints, the emotional burden, and the social burden [34].

Caregiver Stress Self-Test

The Caregiver Stress Self-Test [35] is a self-assessment scale with 14 items that uses a 4-point scale from 0 (never) to 3 (often). A higher score indicates a higher degree of stress. When the score is between 0 and 13 points, the subject is in a good state. When the score is between 14 and 25 points, some signs of stress have begun to appear. When the score is between 26 and 42 points, the burden of stress is greater.

Other scales

Other scales, such as the Distress Management Screening Measure (DMSM), Beck Anxiety Inventory (BAI), Beck Depression Inventory (BDI), Zung's anxiety self-assessment scale (SAS), the Zung depression self-assessment scale (SDS), Profile of Mood States (POMS) Questionnaire, and other scales are also often used in clinical evaluation work.

Existing scales have been tested for their reliability and validity and have been used by many studies to assess the psychological status of caregivers. However, there are still some shortcomings: some assessment tools are simple scales that lack the specific evaluation elements for caregivers, such as BDI, BAI; while some scales are compiled for the group of caregivers, the content of consideration is extremely large. Most of the assessments are the pressure and burden of caregivers, which can only show one aspect of their mental health. The above tools are targeted at all caregivers caring for patients of all diseases; cancer patients are a very special group that needs attention. The specificity of the cancer patients' group causes the psychological distress of their caregivers to be assessed more specifically; some scales have simply been translated and have not been adapted according to China's special cultural background. Pertaining to the application in China, reliability and validity are unknown, and use is limited.

Factors affecting the mental health of cancer caregivers

Related factors for caregivers

General demographic factors of caregivers

Gender The mental health status of caregivers varies. Currently, most studies have shown that women's caregivers have worse mental health. A domestic study [36] found that the incidence of anxiety and depression of female family members was higher than that of males when family members were the primary caregivers. Studies abroad [37] show that the incidence of emotional disorders in female family members (23%) is higher than that of male family members (7%) after two years of cancer diagnosis. However, some scholars [38] believe that the pressure load of male caregivers is higher than that of female caregivers. This is because when men are caregivers, sudden family role changes make it necessary to take care of things that they were not familiar with quickly.

Age In a study of cancer caregivers of patients in hospital chemotherapy [11], it was found that the anxiety and depression level of the main caregivers of cancer showed significant age differences. Middle-aged and elderly caregivers (aged ≥ 45 years) had higher anxiety and depression scores than younger ones (< 45 years). On the other hand, there are studies that called attention to young caregivers. The research results of He *et al* [39] show that with the increase of age, the mental resilience of caregivers has gradually increased, which may be more rich due to the social experience of middle-aged and elderly people, greater maturity in handling matters, and their greater ease in obtaining social support.

Relationship with cancer patients The caregiver can be the patient's spouse, children, other relatives, friends, etc. The relationship between the patient and the caregiver is different, and the mental state of the caregiver will also be different. Studies [11, 40] have shown that the immediate family members' psychological burden during care is higher than that of their other relatives, and the anxiety and depression of parents and spouses are significantly higher than those of their children. Some researchers also believe that the anxiety and depression of patients' spouses and children are significantly higher than other members [41].

Education level The education level of caregivers can to some extent represent their knowledge of disease, their ability to deal with emergencies, their attitudes towards cancer and death, their ability to actively cooperate with treatment, and their social and family economic status. Fleming *et al* [42] suggested that the higher the education level of caregivers, the lower the incidence of depression. Research by Cameron *et al* [43] also found that caregivers with a low educational level have greater psychological stress.

Economic situation Cancer often requires multiple clinical treatments and has a long course. The family financial status of patients not only affects the treatment and prognosis of patients, but it also affects the physical and mental health of caregivers. Studies [11, 36, 44] have shown that caregivers with poor financial conditions have more severe anxiety and depression than those with good financial conditions.

Social support

Taking care of patients over a long period of time tends to reduce the social and entertainment activities of the caregiver. The communication with relatives and friends is also reduced, which easily makes the caregiver feel lonely and abandoned. Studies by Kim *et al* [45] show that the positive feelings of caregivers are positively related to their level of social support, that is, the higher the level of social support of caregivers, the more positive feelings they get. Mosher *et al* [46] pointed out that reduced social support can lead to reduced quality of life for cancer caregivers. In addition, there are studies [47] that show that caregivers' anxiety and depression levels are negatively related to their social support.

Attachment types

Attachment is a strong, lasting emotional connection formed between an individual's early years and his or her main caregivers and plays an important role in the individual's life. Some scholars [48] explored the relationship between the positive feelings of cancer caregivers and attachment and social support, and found that attachment anxiety and social support are the influencing factors of the positive feelings of caregivers; social support plays a mediating role between attachment avoidance and positive feelings.

Hope level

The level of hope is one of the buffering factors that helps caregivers cope with stress, and it is also an important factor that affects the physical and mental health of caregivers [49]. Studies [50] have shown that the total score of the hope level of caregivers of patients with advanced cancer is positively correlated with their quality of life, especially with a moderately positive correlation with the score in the dimension of the psychological field.

Coping style

Coping style refers to the way in which individuals handle stressful situations and are generally divided into positive and negative coping styles. The results of many domestic studies [51–53] showed that positive coping style is an influencing factor of the psychological resilience of cancer caregivers, and the more positive the coping style, the higher the level of psychological resilience. Under the same stress conditions, individuals who took an active response tended to seek outside help and talk to others, which was conducive to alleviating stress and adjusting

their psychological conditions.

Burden

The results of the study [12] indicate that the burden of cancer caregivers is very high. Most cancer caregivers are in a high-burden situation, and the incidence of high-burden conditions (62%) is significantly higher than that of non-cancer caregivers (38%). Grunfled [8] pointed out that the burden of care is the main cause of caregiver anxiety and depression.

In addition, the nature of the care tasks undertaken by caregivers can also affect their psychological status. They believe that difficult or unattractive tasks (such as going to the toilet) may lead to higher levels of pain [54]; research shows that the daily care time [55–56] will affect the quality of life of the caregiver, reduce their rest and social activities, and lead to the emergence of anxiety, depression, loneliness, and other bad emotions for a long time; the existence of medical insurance can also affect the mental health of the caregiver. Insurance can reduce the financial burden of caregivers and relieve their stress [36]; length of care and frequency of care have also been shown to affect psychological resilience [57].

Related factors for cancer patients

Gender and age of patients

Studies [41] have shown that when patients are male, their caregivers' depression is significantly higher. In addition, Turgeon *et al* [57] investigated the mental health of caregivers of cancer patients and found that caregivers of young patients were more likely to have psychological problems.

Cancer stage

As patients experience different stages of their disease, the mental state of their caregiver changes accordingly. A number of studies on the caregivers of cancer patients [8, 57–59] have shown that as the cancer patients' disease progresses, physical function declines, and facing death, the incidence of depression and anxiety of their caregivers will also change. On the other hand, as the patient's condition becomes worse, and the degree of dependence on the caregiver also increases, this increases the care-giving work stress of the caregiver, and then also affects their physical and mental health.

Cancer symptoms

Symptoms of cancer include symptoms caused by the disease itself and the side effects that occur during treatment. The more severe the patient's symptoms, the more time the caregiver needs to spend on care. In a study of cancer patients and their spouse caregivers, Williamson [60] found that the restriction of the caregivers' daily activities mediates the relationship between stress and resentment, and the limitation of activities is predicted by the severity of the patient's symptoms.

Cancer course

There was a positive correlation between the caregiver's mental health and the patient's disease duration. Studies [61] have shown that the longer the patient's disease course, the lower the positive emotions and higher negative emotions of the caregiver, and the more likely they are to cause psychological problems. The results of Liu's study [11] showed that the caregiver's anxiety and depression scores were significantly higher in cancer patients with a course of ≥ 6 months than in those with a course of less than 6 months.

Psychological intervention for cancer caregivers

Caregivers of cancer patients have little preparation, information, or support to perform their care responsibilities. However, their psycho-social needs must be met so that they can maintain their health and provide the best support to their patients. There are meta-analyses [62] of data from 29 randomized clinical trials published from 1983 to March 2009, which provided three types of interventions: psychological education, skills training, and treatment counseling. Most interventions are provided jointly to patients and caregivers. A meta-analysis shows that although these interventions have small to moderate effects, they significantly reduce the psychological burden on caregivers, improve their ability to respond, increase their sense of self-efficacy, and improve all aspects of their quality of life. Various intervention characteristics are also considered as potential regulators. Clinicians need to provide research-proven interventions to help caregivers and patients respond effectively and maintain their quality of life.

Psychological interventions for cancer caregivers are necessary. First, clinicians need to recognize that patients and their home caregivers respond to cancer as a whole. As such, they have a legitimate need to get help from healthcare professionals. There is a general consensus that when patients and their caregivers are treated at the same time, there will be important synergistic effects that will help everyone's health [63]. When the needs of caregivers are not met, their mental and physical health is threatened and patients are denied access to the best care from well-prepared home caregivers [64–65]. Patient-only care plans rarely meet the needs of patients because patient care relies heavily on caregivers. To improve the level of comprehensive cancer care, patients and caregivers should be taken care of when implementing a care plan. Second, there is clear evidence [63] that interventions provided to caregivers of cancer patients can positively affect many aspects of caregivers. Interventions significantly reduced the burden on caregivers, improved their coping skills,

increased their confidence as caregivers, reduced their anxiety, and improved marriage and family relationships. These interventions appear to prepare caregivers and reduce their suffering, which is likely to have a substantial positive impact on patients.

Different schools have different ways of performing psychological interventions. In clinical work, the common psychological intervention methods are as follows:

Cognitive-behavior therapy (CBT)

CBT is a structured, short-range, current-oriented approach to psychotherapy developed by A. T. Beck. It is an effective measure to improve the quality of life of the caregiver and reduce the degree of psychological distress by changing the mindset and behavior in order to correct irrational cognition, thereby reducing the negative mood and behavior. As a positive psychological treatment method, CBT has been used in psychological interventions for caregivers of cancer patients, and research [66] shows that CBT can alleviate the depression and improve the quality of life of caregivers of cancer patients. Research by Tang *et al* [67] showed that cognitive behavioral intervention can effectively reduce the anxiety, depression, and care burden of primary caregivers of patients with chemotherapy for bowel cancer, and can effectively improve their level of positive coping styles. Research by Chen *et al* [68–69] showed that CBT can effectively reduce the care burden of the primary caregivers of patients with lung cancer undergoing chemotherapy and improve their quality of life. The research by Qin *et al* [70] showed that the implementation of CBT can effectively improve the post-traumatic growth and quality of life of caregivers of patients with oropharyngeal cancer, allow caregivers to play a more effective role, and improve the quality of care. In addition, CBT can also improve the mental health level for caregivers of patients with dementia [71], brain surgery [72], schizophrenia [73], stroke [74], and Alzheimer's disease. After interventions, both patients' [75] and caregivers' [76] mental health improved.

Mindfulness-based stress reduction (MBSR)

MBSR is a psychological intervention method founded by Jon Kabat-Zinn. The goal of mindfulness is to remain aware at all times to free yourself from a strong attachment to faith, thought, or emotions, thereby developing a greater sense of emotional balance and happiness. One of the purposes of mindfulness practice is to take greater responsibility for your life choices. Therefore, mindfulness can optimize health prevention and disease recovery by participating in and strengthening an individual's internal resources. For refractory diseases, meditation techniques can alter and improve consciousness, regulate the subjective experience of pain, or improve the ability to manage pain or disability [77]. Xu's study [78] showed that MBSR is a safe, simple, and convenient method that can reduce the level of anxiety and depression of caregivers of patients with malignant tumors. Lengacher *et al* [79] conducted a six-week mindfulness decompression intervention for cancer patients and their caregivers, and the caregivers' psychological condition and quality of life improved, while also reducing the patient's tension and anxiety. In addition, studies have shown that MBSR can improve the negative emotions of the family members of patients with depression and adjust their coping styles [80]. It can effectively reduce the burden of care of the primary caregivers of patients with severe head injury and improve the psychological flexibility of the primary caregivers, thus increasing their positive coping style and positive experience [81]. It can also improve the negative mood of caregivers of schizophrenic patients and strengthen their self-esteem [82].

Music therapy

Music therapy is based on the theory and methods of psychotherapy and uses the unique physiological and psychological effects of music to enable patients to undergo the musical experience through various specially designed musical behaviors, with the participation of music therapists, to eliminate psychological barriers. The purpose of restoring or improving mental and physical health is divided into active music therapy, passive music therapy, and comprehensive therapy. Research by Chen *et al* [83] showed that music therapy can effectively assist analgesia after finger replantation; Ye's research [84] showed that music intervention can improve the anxiety and depression of the family's main caregiver in patients with acute myocardial infarction. At the same time, it can also improve the symptoms of the digestive system. Studies abroad have shown that the intervention of Turkish classical music on patients with dementia at home can reduce the nursing burden of the nursing staff and control the blood pressure of patients [85]. In addition, studies [86] have shown that music therapy in combination with other psychological interventions improves the mental health of caregivers.

Cancer not only causes physical and psychological pain to patients, but also challenges their caregivers. Compared with patients, the psychological pain of caregivers is often overlooked. However, in recent years, medical professionals and related scholars have gradually realized the need to include care for caregivers as part of the cancer care system. More accurately assessing the psychological pain of caregivers of cancer patients, and conducting scientific, timely, and reasonable psychological interventions can not only improve the mental health of cancer caregivers, but also indirectly

improve their level of care, which is more conducive to the prognosis of cancer patients.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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CASE REPORT

Gastric cancer with soft tissue metastasis of the abdominal wall: a case report

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Abstract

Abdominal wall metastasis of gastric cancer (GC) is a very rare occurrence in the clinic setting. We recently diagnosed and treated a patient with abdominal wall metastasis of GC and we hope to provide some helpful guidance on the clinical diagnosis and treatment of this disease.A 49-year-old male patient with GC was admitted to our hospital (Dalian Municipal Central Hospital, Dalian, China) complaining of left upper abdominal wall mass. Physical examination and regular laboratory blood tests showed no obvious abnormalities. Ultrasound and CT of the abdomen showed a subcutaneous solid mass in the abdominal wall. Radical gastrectomy was performed on February 27, 2019, six months after it was first noticed by the patient. Pathological examination and immunohistochemistry showed GC with abdominal metastasis. Postoperative radiotherapy or chemotherapy was not pursued after the second operation and no recurrence or metastasis has been noted so far. GC with abdominal metastasis is very rare and can be easily missed or misdiagnosed. For metastasis to a single site in the abdominal wall, surgical resection, which is recommended, may improve patient outcomes.

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Key words: soft tissue; abdominal wall; metastasis; gastric cancer (GC)

Gastric cancer (GC) is one of the most common malignant cancers worldwide [1]. In China, it is the second most common cancer causing morbidity and the third most common cancer causing mortality [2]. Despite the advances in both diagnosis and therapy, most patients die because of recurrence and metastasis after surgery and/or radiochemotherapy. The common sites for metastasis include the liver, lung, and bone. GC with abdominal metastasis is a very rare occurrence. We recently reported a case of GC with soft tissue metastasis to the abdominal. We hope to provide some helpful guidance on the clinical diagnosis and treatment of this disease.

Case report

A 49-year-old male with a 3 weeks history of epigastric pain was admitted to the Dalian Municipal Central Hospital, Dalian, China, on February 22, 2019. His epigastric pain was dull, intermittent, and tolerable. During this period, the patient had no nausea, vomiting, diarrhea, abdominal distention, dizziness, or weakness. Gastroscopy showed a deformation leading to narrowing of the antrum. A large amount of residual food was noted in the stomach. The fundus, body, and duodenum were unremarkable. Biopsy of the deformed gastric antrum was performed and the pathology results were indicative of an adenocarcinoma. His routine blood exam was significant for hemoglobin of 136 g/L; carcinoembryonic antigen of 9.39 ng/mL, carbohydrate antigen 19-9 of 232.10 U/mL. The liver and kidney functions were normal. On CT, the liver, lung, and brain were unremarkable.

On February 27, 2019, radical resection of the tumor was performed. Intraoperatively, the mass, about 8.0 cm × 6.0 cm, was located in the antrum, and was noted to be hard and infiltrating the serosa. The postoperative pathological results were as follow: (gastric) ulcerative type of moderately differentiated adenocarcinoma, part of which was mucinous adenocarcinoma (tumor size 3.5 cm × 2.5 cm × 1.5 cm, Lauren's type: intestinal type; Fig. 1). The tumor cells invaded blood vessels and nerves as

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well as the entire serosa and surrounding fibrous adipose tissue. No tumor cells were noted on both sides of the resection edges. Lymph node metastasis in the large and small curvatures was noted (1/3 and 1/12). After the resection, chemotherapy with SOX regimen (oxaliplatin combined with tegio) was administered for six cycles.

On August 3, 2019, the patient inadvertently found a mass on the left upper abdominal wall, the size of the nail plate. This new mass was hard, had unclear boundaries, and had ill-defined activity. The patient had no swelling, pain, or ulceration of the local skin. Ultrasound of the abdominal wall showed a subcutaneous solid mass, about 2.1 cm × 1.1 cm in size, 0.2 cm from the body surface, with clear boundaries, irregular in shape with spot blood flow signals around and inside the mass (Fig. 2a). Abdominal CT showed a mass of about 2.5 cm × 1.0 cm in size in the left upper abdominal wall, irregular in shape and with unclear boundaries (Fig. 2b). No metastasis to the liver, lung, and brain was noted on CT exam. The patient and his family members refused a biopsy.

On August 9, 2019, another surgical resection was performed. The tumor and 2 cm of surrounding tissue were completely resected. The pathological exam was indicative of adenocarcinoma (Fig. 3). Immunohistochemistry (IHC) was positive for CDX-2, CK20^{+}, MUC-2, and MUC-6 (Fig. 4). Based on the postoperative pathology report and IHC, GC with metastasis to the abdominal wall was diagnosed. Postoperative recovery was unremarkable. The patient and family refused radiotherapy and chemotherapy after operation. As of now, there has been no recurrence or new metastasis on follow-up visits.

Discussion

GC is one of the most common malignant tumors in the digestive tract. Metastasis can occur via direct invasion, lymphatic spread, hematogenous spread, or by peritoneal implantation. Common metastatic sites include liver, lung, bone, brain, and adrenal. However, metastasis to the abdominal is a very rare occurrence in the clinic setting and the mechanism of metastasis is still unclear. At present, there are only a few studies on GC with metastasis to the abdominal wall. Also, there is a lack of relevant experience in the diagnosis and treatment of GC with metastasis to the abdominal wall.

There are no defining clinical manifestations of GC with metastasis to the abdominal wall. The only presenting sign is an abdominal wall mass, difficult to be associated with a primary malignancy of gastric origin. Therefore, it is very important to select the appropriate examination. Currently, ultrasonography, MRI, and CT are the modalities used for auxiliary examination of soft tissue masses in the abdominal wall; however, definitive diagnosis requires puncture biopsy or resection

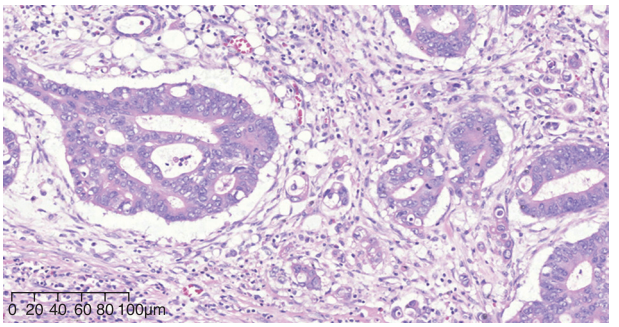

Fig. 1 HE staining showing gastric mass was a moderately differentiated adenocarcinoma

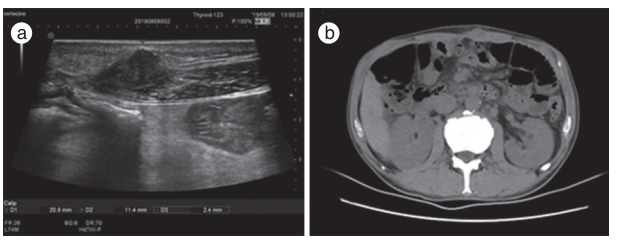

Fig. 2 Ultrasound (a) and CT (b) showing a soft tissue tumor in the left upper abdominal wall

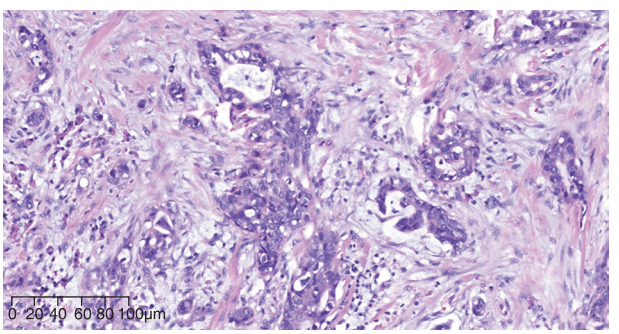

Fig. 3 Pathology of the soft tissue mass showing a metastatic adenocarcinoma

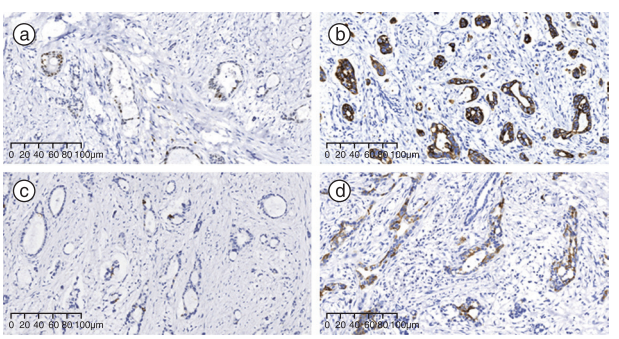

Fig. 4 IHC of the soft tissue mass. The neoplastic cells were positive for CDX-2 (a), CK20^{+} (b), MUC-2 (c), and MUC-6 (d)

followed by pathological examination. Ultrasonography is the first-choice modality for imaging abdominal wall tumors. It can directly and clearly show the anatomical location, scope, size, depth, tumor boundaries, and the relationship with surrounding tissues. At the same time, it can demonstrate internal blood flow in tumors. Most of the malignant tumors have a rich blood flow signal, a high density of blood vessels and a high-speed and low resistance arterial blood flow signal mostly located at the edge of the tumor [3]. CT can better show the relationship between the tumor and the surrounding tissue structure. In this case, the solid abdominal wall mass could be seen by ultrasonography, with clear boundaries and an irregular shape. Also, spot blood flow signals could be seen around and inside of the abdominal wall; CT examination indicated the mass with an irregular shape and unclear boundary located in the left upper abdominal wall. However, at present, benign and malignant tumors of the abdominal wall cannot be distinguished based solely on imaging studies [4]; only biopsy or postoperative pathological findings can provide a definitive diagnosis. Also, IHC can help identify the source of the tumor. In our case, GC with metastasis to the abdominal wall was diagnosed based on the combination of a history of GC and the results from IHC. Therefore, although the incidence of GC with metastasis to the abdominal wall is relatively low, a detailed history should be obtained from patients presenting with a mass on the abdomen, especially if subsequent imaging examinations indicate the possibility of malignancy. To avoid misdiagnosis or missed diagnosis, clinicians should pay more attention to any previous history of malignancy.

At present, there are a few studies on the treatment of GC with metastasis to the abdominal wall. There is no standard treatment, however, the principle of treatment for patients with a single or several metastatic foci limited to one location is the removal of the primary focus and is the gold standard treatment. This treatment modality can improve prognosis. For abdominal wall cancer, postoperative radiotherapy may play an important role in killing residual tumor cells and preventing recurrence. A retrospective randomized controlled study showed that radiotherapy can effectively reduce the local recurrence rate of soft tissue tumors, but it had no significant impact on the overall survival rate. For highly differentiated soft tissue tumors, external radiotherapy and brachytherapy are effective, while for poorly differentiated soft tissue tumors, only external radiotherapy is effective [5]. If the primary focus cannot be controlled or there is metastasis to multiple sites, systemic treatment such as symptomatic treatment and chemotherapy can improve the quality of life of patients, but the overall prognosis of such patients is poor [6]. Jiang *et al* reported a patient with GC with soft tissue metastasis to the left lateral thigh [7]. The patient was treated with interventional embolization and was given oxaliplatin + tegio chemotherapy for six cycles. Finally, the patient had metastasis to multiple sites including the liver and lung and subsequently died of multi-organ failure after 12 months [7]. Zhang reported a patient with GC with soft tissue metastasis of the right lower extremity. The patient refused tumor resection and left the hospital after one cycle of chemotherapy with FAM regimen; the remainder of his course remains unknown [8]. In our case, the primary focus of GC was removed and the tumor in the abdominal wall was completely removed. The patient and his family refused radiotherapy and chemotherapy after the second operation. At present, the patient has had no recurrence or metastasis.

GC with metastasis to the abdominal wall is a very rare occurrence and can be easily missed or misdiagnosed in the clinic setting. In GC with metastasis to a location in the abdominal wall or with several metastatic foci limited to one location, surgery, which may improve patient outcomes, is recommended. Postoperative radiotherapy and chemotherapy are recommended as well.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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CASE REPORT

Modified Appleby operation for carcinoma of the pancreatic body and tail

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Abstract

This is a case of a pancreatic tumor with invasion of the celiac stem treated using the modified Appleby operation. Preoperatively, routine B-ultrasound, computed tomography, and magnetic resonance imaging were performed. In the perioperative period, the combined gallbladder was excised; the duration of the operation was 5 h and volume of blood loss was approximately 500 ml. Postoperatively, the liver function temporarily returned and after a liver protection treatment, it returned to normal within 2 weeks. The liver had normal arterial blood supply, and the postoperative course was uneventful. It is safe and feasible to resect the whole pancreatic body and tail tumor combined with celiac stem resection. It can improve the resection rate of tumor and relieve pain.

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Key words: modified Appleby operation; pancreas cancer

Clinical data

A 68-year-old patient was admitted to the hospital on February 13, 2019, owing to complaints of epigastric pain and discomfort with anorexia that started more than two months previously. On physical examination, the abdomen was flat and soft, with left epigastric tenderness, no rebound pain, and no palpable mass. On auxiliary examination, abdominal B-ultrasound showed pancreatic space-occupying lesions and multiple gallstones. Thin slice computed tomography (CT) plain scan and enhanced scan of the pancreas showed that the shape of the pancreas was irregular and an irregular soft tissue density mass was found in the body of the pancreas, measuring approximately 2.5 cm × 2.3 cm with an unclear boundary and uneven density; the enhanced scan showed an opposite low density, atrophy of the tail of the pancreas, dilatation of the pancreatic duct, and a close relationship between the lesions and splenic artery and celiac trunk. It was consistent with the characteristics of pancreatic cancer, considering the invasion of the splenic artery and celiac trunk, and that multiple gallstones were found in the gallbladder. Magnetic resonance imaging plain scan and enhanced scan of the whole abdomen (Fig. 1) revealed that a nodular abnormal signal on both T1- and T2-weighted images could be seen in the body of the pancreas, with a high signal on DWI, measuring 19 mm × 20 mm. After enhanced scanning, the arterial phase enhancement was not evident, the volume of the tail of the pancreas was reduced, and the distal pancreatic duct was expanded; therefore, it was considered as pancreatic body cancer because the multiple gallstones were found in the gallbladder after exploring the swelling. The tumor indices were as follows: AFP 1.52 μg/mL; CEA 3.90 μg/L, CA 1993.19 μ/mL; and the modified Appleby operation was performed under general anesthesia on February 21, 2019. During the surgery, the common hepatic artery in the proximal and distal parts of the gastroduodenal artery and its origin were exposed freely, and the common hepatic artery was clamped. The pulse of the artery continued to remain palpable in the proper hepatic artery; therefore, we decided to perform the modified Appleby operation. The main hepatic artery was cut off near the origin of the gastroduodenal artery. Thereafter, the whole body and tail of the pancreas and spleen including the tumor were cut off from the neck of

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the pancreas. The right omental and gastric arteries were preserved to maintain the blood supply to the stomach. The gallbladder was removed during the operation. The duration of the operation was 5 h, and the volume of blood loss was approximately 500 mL. Postoperatively, the liver function temporarily returned and after a liver protection treatment, the liver returned to normal within 2 weeks. The liver had normal arterial blood supply, and the postoperative course was uneventful. Pathological examination revealed differentiated adenocarcinoma of the pancreas with nerve invasion (Fig. 2). There was no recurrence or metastasis at 1, 3, and 6 months after the surgery.

Discussion

The first Appleby operation in the world was performed in 1953, which included resection of the whole stomach, body and tail of the pancreas, the spleen, and combined resection of the celiac trunk and common hepatic artery. In 1976, Nimura *et al* [1], School of Medicine of Nagoya University, Japan, applied Appleby operation to the extended radical operation of the body and tail of the pancreas for the first time. Owing to the removal of the abdominal trunk, the blood supply to the liver and stomach was seriously affected. In 1991, Hishinuma *et al* [2] carried out the first modified Appleby radical resection of pancreatic body and tail carcinoma with preservation of the stomach. It is suitable for patients with no tumor invasion at the root of the celiac trunk, with common hepatic artery (CHA) and bifurcation of the gastroduodenal artery (GDA), and in those in whom the tumor invades the celiac trunk but not the superior mesenteric artery (SMA) and proper hepatic artery (HA).

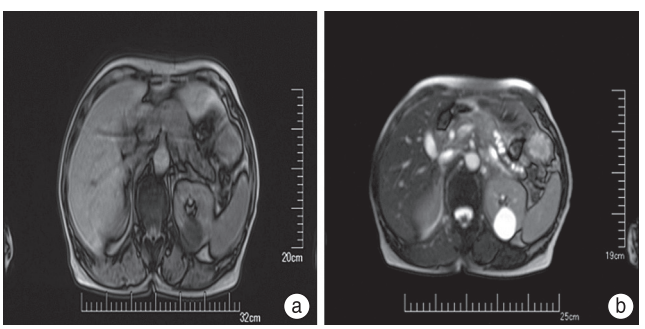

Fig. 1 (a) Magnetic resonance imaging (MRI) cross-sectional plain scan. Nodular abnormal signal on T1 and T2-weighted images seen in the body of the pancreas. (b) On the cross-sectional enhanced MRI scan, the arterial phase enhancement is not evident, the volume of the pancreatic tail is decreased, and the distal pancreatic duct is dilated

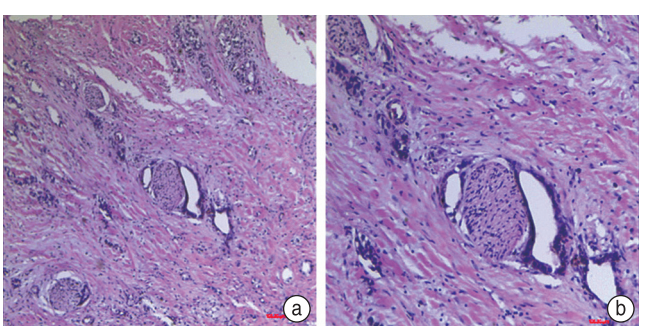

Fig. 2 Postoperative pathological examination reveals tumor cells to be arranged in an irregular glandular tube with infiltrating growth; the cells are atypical. (a) HE staining × 50; (b) HE staining × 100

The anatomical basis of the improved Appleby operation is that the CHA sends out the proper HA and the gastroduodenal artery [3], and the upper pancreaticoduodenal artery from the gastroduodenal artery and the lower pancreaticoduodenal artery from the SMA form the pancreaticoduodenal artery arch around the head of the pancreas; therefore, when the CHA is cut off, the arterial blood can flow from the SMA through the 12 fingers of the pancreas. The intestinal arch and gastroduodenal artery are injected into the proper HA to ensure blood supply to the liver.

The indications of the modified Appleby operation were as follows: (1) the tumor was confined to the tail of the pancreas without invasion of the head of the pancreas and without distant metastasis; (2) the tumor did not invade the proper HA and the SMA; (3) the root of the celiac trunk and the bifurcation of the common hepatic and gastroduodenal arteries were not infiltrated by the tumor, and therefore, the celiac trunk could be ligated and cut off at the root, and the bifurcation of the common hepatic and gastroduodenal arteries could also be cut off. The main HA was ligated and cut off on the central side of the liver; (4) the retroperitoneal tumor could be removed completely during the operation; and (5) the main HA could be blocked experimentally during the operation, and the pulsation of the inherent HA could be evidently noted after 1–2 minutes, implying that even if the main HA was cut off, the body could maintain sufficient blood flow to the HA [4].

The key to improve the Appleby operation is to determine the integrity of the pancreaticoduodenal artery arch by preoperative celiac trunk and SMA angiography. In addition to routine angiography, a balloon catheter can be used to temporarily block the CHA, and then SMA angiography can be used to determine whether the artery arch is developed or if coil embolization can be used to make the pancreaticoduodenal artery arch compensatory expansion, or if 3D reconstruction technology of preoperative blood vessel CT can be used to understand the relationship between the tumor and blood vessels [5]. During the operation, the CHA can be temporarily blocked and the inherent HA can be examined for pulsation; Color Doppler ultrasound was used to evaluate

the blood flow of the proper HA after the occlusion of the CHA.

Advantages of improved Appleby operation: (1) It improves the resection rate of pancreatic cancer, thereby providing patients with a longer survival period [6]. The traditional view is that the invasion of the celiac stem by pancreatic cancer is an unresectable disease; if it cannot be removed, the average survival period is 6–10 months, and the 5-year survival rate is less than 5%. The average survival period of patients after the modified Appleby operation can be increased until 21 months. The 5-year survival rate can be increased until 25%. (2) It improves the quality of life of patients after surgery, mainly alleviating intractable abdominal and back pain, which can be relieved immediately and made last for a long time.

Disadvantages of the improved Appleby operation: Although the improved Appleby operation does not need reconstruction of the blood vessels and digestive tract and the perioperative mortality is low, the postoperative complications rate is as high as 48%, including postoperative liver function abnormality, liver abscess, gallbladder necrosis, gastric mucosal ischemia, gastric ulcer formation, and diarrhea. The postoperative liver function abnormality is mostly transient, which can be reduced to normal several days after the surgery [7]. Gallbladder necrosis is a fatal complication. Some experts believe that the modified Appleby operation requires routine cholecystectomy. This patient was complicated with multiple gallbladder stones, chronic cholecystitis, and evidence of cholecystectomy. Most of gastric mucosal ischemia and ulcer formation can be alleviated by acid suppression therapy. Experts believe that embolization of the CHA before operation can promote the collateral circulation of SMA and protecting the vessels of the lesser curvature of the stomach during the operation can reduce this complication. In addition, because the celiac plexus was excised at the same time, it is easy to have persistent diarrhea. The incidence of postoperative diarrhea was 62.5%, of which 75% needed to be administered antidiarrheal drugs for a long time. There were no serious postoperative complications in this patient.

To sum up, there are only a few reports about the modified Appleby operation in the treatment of advanced pancreatic cancer, and most are case reports. Some reports also expand the scope of surgical resection. Owing to the limited number of cases, whether patients can benefit from the operation is still controversial. Although some studies have confirmed that the modified Appleby procedure is safe, effective, and feasible in the treatment of advanced pancreatic body and tail cancer, larger cohort, multicenter case-control studies are lacking. Patients should undergo a routine multidisciplinary discussion before the operation, and it is recommended that experienced pancreatic surgeons should perform the procedure in larger pancreatic centers.

Ethics approval and consent to participate

This report was approved by the Institutional Review Board and Human Ethics Committee of the Affiliated Huaian No. 1 People's Hospital of Nanjing Medical University (Huaian, China). The patient provided a written informed consent to participate.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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