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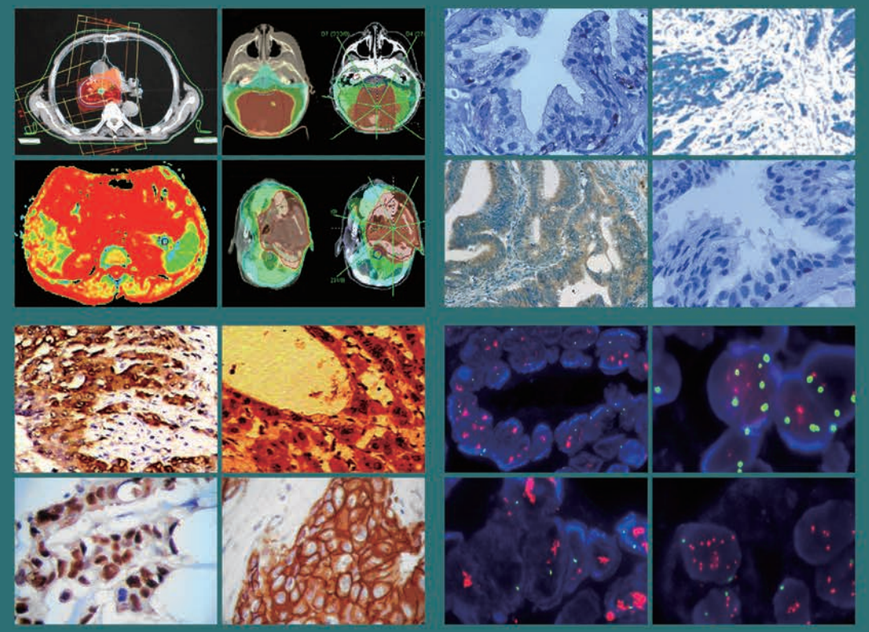

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REVIEW ARTICLE

Targeted therapy of gastric cancer: current and prospective strategies*

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Abstract

Gastric cancer is the third leading cause of cancer-related death worldwide. Surgery is currently the only curative treatment strategy. Chemotherapy has shown limited efficacy in advanced gastric cancer patients with a median overall survival of less than one year. Thus, new treatments are urgently needed. Trastuzumab and Ramucirumab are the only targeted therapies approved currently. Most Phase III clinical trials evaluating targeted drugs in gastric cancer have failed. This review will evaluate relevant clinical trials with targeted therapies performed in gastric cancer patients, discuss the possible reasons for the failure, and indicate new possibilities to enhance gastric cancer treatment.

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Gastric cancer is the third most common cause of tumor-related death globally, with about one million new cases diagnosed each year [1]. The epidemiology of gastric cancer has distinct regional differences: the highest mortality rate is in East Asia, and the lowest is in North America; non-cardia gastric adenocarcinoma is commonly seen in East Asia, Eastern and Central Europe, Latin America, and Africa, whereas gastroesophageal junction gastric adenocarcinoma and proximal gastric cancer are commonly seen in in Western Europe, North America, and Australia [2]. Surgery is the most important method for the comprehensive treatment of localized gastric cancer, but chemotherapy is the only standard treatment for recurrent or metastatic gastric cancer [3]. The median survival time of patients with unresectable gastric cancer is 8–11 months in Europe [4–5] and 13–16 months in East Asia [6–7].

To date, international large-scale oncogenomic studies have given detailed molecular typing on gastric cancer. The Cancer Genome Atlas (TCGA) project analyzed 295 primary gastric cancer tissues not subject to radiotherapy and chemotherapy, and classified gastric cancer into four subtypes: EBV positive (EBV), microsatellite instability (MSI), genomic stability (GS), and chromosomal instability (CIN) [8]. EBV-positive gastric cancer mostly takes places at the gastric fundus and gastric body, often concomitant with P1K3CA or ARID1A hypermutation, DNA hypermethylation, and high expression of PDL1/PDL2; MSI subtype gastric cancer is usually accompanied by a high mutation rate and hypermethylation; GS subtype gastric cancer is often associated with molecular change in the pathways relevant to cell adhesion and metastasis and ARID1A and RHOA mutations; CIN subtype gastric cancer is usually accompanied by mutation of TP53 and gene amplification of receptor tyrosine kinases (RTKs). Although this classification has no prognostic value, it provides a basis for the selection of gastric cancer therapy. The most recent molecular typing of gastric cancer adopted by the Asian Cancer Research Group (ACRG) is similar to that adopted by the TCGA—both containing the MSI subtype, but the MSS subtype adopted by ACRG is further divided into smaller subgroups according to the EMT and TP53 conditions. The MSS/EMT subtype has the worst prognosis and a high recurrence rate, whereas the MSI subtype has the best prognosis [9]. In general, these studies have deepened our understanding of the molecular mechanisms of gastric cancer and facilitated personalized therapy of this cancer.

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With the advancement of molecular biology research on gastric cancer, the demand for personalized therapy based on its molecular biology has emerged. The exploration of new, targeted therapeutic drugs and their joint application with other therapeutic tools is an urgent issue that should be addressed by researchers. This article elaborates on the current progress in targeted gastric cancer therapy, such as anti-EGFR, HER2, VEGF, and mTOR treatments, and suggests future strategies of targeted gastric cancer therapy.

EGFR inhibitors

Human epidermal growth-factor receptors (HER) are a family of tyrosine kinase receptors, including EGFR/HER1, HER2/neu, HER3, and HER4 [10]. After binding with ligands, EGFR transforms into a homo- or heterodimer to activate the EGFR pathway and further affect multiple cellular physiological processes, including proliferation, adhesion, invasion, migration, and differentiation [11]. About 5% of gastric cancer patients show EGFR amplification with poor prognosis [8]. It has been confirmed by some studies that EGFR is an effective therapy target for tumors.

Cetuximab

Cetuximab is an IgG monoclonal antibody that binds to the extracellular domain of EGFR and prevents ligand binding to EGFR. A cetuximab-based prospective, multi-center phase II clinical trial showed that patients diagnosed with advanced gastric cancer or gastroesophageal junction adenocarcinoma but not previously treated, when treated with cetuximab in conjunction with cisplatin and capecitabine in the trial, had an objective response rate (ORR) of up to 53.2%, a median progression-free survival (PFS) time of 5.2 months, and a median overall survival (OS) time of 10.8 months [12]. However, the EXPAND trial showed that in patients already treated with both cisplatin and capecitabine, the combined use of cetuximab with them did not provide any additional benefit [13]. The REAL3 trial also confirmed that patients with advanced gastric cancer cannot benefit from anti-EGFR antibodies [14]. It has been experimentally confirmed that the effect of cetuximab is significantly related to EGFR amplification or high expression [15]. Similarly, a phase II clinical trial also confirmed that the effect of cetuximab in combination with chemotherapy is related to the number of EGFR amplifications [16]. However, in the EXPAND trial, there was no clear relationship between the immunohistochemistry score of EGFR and effect of cetuximab, and the relevant mechanism should be further researched.

Nimotuzumab

Nimotuzumab is the first humanized monoclonal antibody that specifically binds EGFR. A phase II clinical trial of nimotuzumab enrolled 34 patients of recurrent or metastatic advanced gastric cancer, with the control group subject to standard DCF chemotherapy and the observation group subject to a joint treatment by DCF and Nimotuzumab, and found that the PFS and OS were 4.50 and 8.25 months in the control group, respectively, whereas they reached 6.50 and 12.50 months in the observation group, respectively; the difference was statistically significant, and there were no significant differences in toxicity and side effects between the two groups [17]. Another phase II clinical trial (NCT02370849) examined whether the use of nimotuzumab could benefit patients with advanced gastric cancer who initially received cisplatin-S1 chemotherapy and has been almost completed by now, with a result expected imminently. Japanese and Korean researchers initiated a phase III clinical trial (NCT01813253), in which the enrolled patients were those in a: first-line treatment group diagnosed with advanced or recurrent gastric cancer or with a gastroesophageal junction tumor and EGFR over-expression following the failure of 5-FU and cisplatin-based chemotherapy; second-line treatment group (control group) treated with irinotecan monotherapy; an observation group treated with both irinotecan and nimotuzumab, and this study is still recruiting suitable patients.

HER2 inhibitors

HER2 is a member of the EGFR family, and different degrees of HER2 amplification have been observed in various tumors including breast, gastric, colorectal, and lung cancer [18]. The HER2 positive ratio of gastric cancer in different studies is mostly between 6%–30% [19–21]. FISH-positive gastric cancer tissues that also show HER2 overexpression (HER2 3+ or HER2 2+) detected with immunohistochemistry can be defined as HER2-positive tissues. Unlike breast cancer, HER2 expression in gastric cancer tissues is significantly heterogeneous, and therefore, the criteria for the determination of HER2 positivity are not the same. Many preclinical studies and clinical trials have confirmed the importance of HER2 status for targeted gastric cancer treatment.

Trastuzumab

Trastuzumab is the first targeted drug approved for the treatment of gastric cancer. It can specifically bind to the HER2 extracellular domain. As a phase III clinical trial, the ToGA test is a milestone regarding the role of trastuzumab in gastric cancer treatment. In this trial, 594 patients with HER2-positive gastric cancer were

enrolled and randomly assigned to a group treated with chemotherapy alone and a group treated with both chemotherapy and trastuzumab [7], and the results showed that OS in the latter group was 2.7 months longer than that in the former group. Therefore, the results of this clinical trial were cited in the NCCN guideline in 2013, and to date trastuzumab has been recommended for first-line treatment of HER2-positive advanced gastric cancer. A second-phase clinical trial in South Korea studied the effect of the combined use of trastuzumab and XELOX in the treatment of HER2-positive advanced gastric cancer, with an ORR of 67% and a median PFS and OS of 9.8 and 21.0 months, respectively; the most common grade 3–4 toxic side effects were neutropenia (18%), anemia (11%), and peripheral neuropathy (11%). The results of this clinical trial suggested that the combined use of trastuzumab and XELOX is a tolerable and effective treatment method for advanced gastric cancer [22]. In addition, phase II clinical trials in Singapore and Japan confirmed that the treatment with both trastuzumab and S1-cisplatin/docetaxel was also effective for HER2-positive advanced gastric cancer [23–24].

Pertuzumab

Pertuzumab is also a monoclonal antibody that specifically blocks the HER2 pathway. Monotherapy with pertuzumab has limited efficacy, but pertuzumab shows a synergistic effect with trastuzumab in HER2-positive breast cancer that progresses after trastuzumab treatment [25]. Currently, relevant clinical trials are investigating the role of the combined use of pertuzumab, trastuzumab, and chemotherapy in the treatment of gastroesophageal junctional tumors and gastric cancer, and in particular the INNOVATION (NCT02205047) trial is studying the efficacy of this regimen in new adjuvant therapy, whereas the JACOB (NCT01774786) trial studied the role of this regimen in the therapy of metastatic tumors and the results have not been reported.

T-DM1

T-DM1 is a novel, targeted drug that couples trastuzumab with the anti-microtubule drug maytansine and has been approved for HER2-positive metastatic breast cancer. Preclinical studies have found that T-DM1 is effective in HER2-positive gastric cancer models [26]. Unfortunately, a randomized, open-label, phase II/III clinical trial has shown that T-DM1 is not superior to taxanes when used to treat the patients diagnosed with advanced HER2-positive gastric cancer and have been previously treated [27], which is a negative result with unknown reason, but may be interpreted by that after first-line treatment with both chemotherapy and trastuzumab, the HER2 status of some patients with gastric cancer changed, thereby affecting T-DM1 efficacy.

Lapatinib

Lapatinib is a small-molecule tyrosine kinase inhibitor (TKI) that effectively inhibits HER2 kinase and slightly inhibits EGFR kinase. In the SWOG-S0413 phase II clinical trial, lapatinib monotherapy was used for first-line treatment of HER2-positive advanced or metastatic gastric cancer. However, this clinical trial did not reach the expected endpoint, with an ORR of only 11% and median OS 4.8 months [28]. A subsequent European Phase II clinical trial compared the efficacy of lapatinib and lapatinib with capecitabine in second-line treatment of HER2-positive gastric cancer, and only 2 of 37 enrolled patients showed objective relief, resulting in early closure of this clinical trial [29]. A phase III TRIO-013/LOGiC clinical trial found that the effects of the combined use of capecitabine and oxaliplatin–with or without lapatinib–on OS of patients with HER2-positive gastric cancer are not significant, but combined use of the former two with lapatinib can significantly prolong PFS of the patients [30]. TyTAN Phase III clinical trials have confirmed that administration of paclitaxel as a second-line treatment of HER2-positive advanced gastric cancer–with or without administration of lapatinib–does not provide survival benefits to patients [31], a result different from the positive results obtained in patients with breast cancer. A number of clinical trials are still ongoing.

Afatinib

Afatinib has irreversible inhibitory effects on EGFR and HER2 tyrosine kinases and is approved for advanced non-small cell lung cancer and HER2-positive advanced breast cancer. Clinical trials on the effects of the combined use of afatinib and trastuzumab on HER2-positive metastatic refractory esophageal and gastric cancer are ongoing, and current data show that after four months of afatinib monotherapy, the disease control rate reached 42%, whereas the results of the combined use of the two drugs have not yet been reported.

VEGF inhibitors

Angiogenesis can provide nutrients and oxygen to tissues while excreting metabolic waste and carbon dioxide, and has been considered one of the important causes resulting in tumor progression [32]. Tumor tissues secrete angiogenesis-related growth factors, such as VEGF, bFGF, and regulate angiogenesis by binding to receptors on the surface of epithelial cells. Binding of VEGF with the extracellular domain of its receptor phosphorylates its intracellular domain to activate the downstream pathway. Anti-vascular therapy is one of the important methods to treat cancer.

Bevacizumab

Bevacizumab is a humanized IgG1 monoclonal antibody that binds to VEGF and inhibits the VEGF/VEGFR signaling pathway. A phase II clinical trial with 35 patients investigated the efficacy of capecitabine, oxaliplatin, and bevacizumab in metastatic esophageal cancer and gastric cancer, and found that the median PFS and OS were 7.2 and 10.8 months, respectively, the response rate was 51.4%, and drug-related toxicity was tolerable [33]. The AVAGAST trial enrolled 774 patients with advanced gastric cancer who were treated with either 7.5 mg/kg bevacizumab or placebo and simultaneously received chemotherapy with both cisplatin and capecitabine, and found that the median OS and PFS for the bevacizumab patients were 12.1 and 6.7 months, respectively, whereas they were 10.1 and 5.3 months in the placebo group, respectively, indicating that the combined treatment with bevacizumab and chemotherapy significantly prolonged PFS and increased ORR, but the clinical trial did not reach its primary endpoint OS [34]. Recently, Shen *et al* initiated a randomized, double-blind phase III clinical trial, in which Chinese patients with inoperable locally advanced or metastatic gastric cancer were treated with both bevacizumab and capecitabine-cisplatin. A total of 202 patients (102 in the placebo group and 100 in the bevacizumab group) were enrolled in the clinical trial. The results showed that there was no significant difference in OS and PFS between the two groups, and the patients could tolerate the treatment with both bevacizumab and capecitabine-cisplatin [35].

Ramucirumab

Ramucirumab is a novel IgG1 monoclonal antibody that specifically binds to the extracellular domain of VEGFR2 and inhibits the VEFGR2-related pathway. The REGARD trial (NCT00917384) enrolled patients with metastatic gastric or gastroesophageal junctional tumors who had received an unsuccessful platinum- or fluorouracil-based first-line treatment. A total of 335 patients were randomly divided–at a ratio of 2:1–into a ramucirumab group (8 mg/kg/2 weeks) and a placebo group, and all the patients received best support treatment. Median OS and PFS were 5.2 and 2.1 months in the ramucirumab group, respectively, whereas they were 3.8 and 1.3 months in the placebo group, respectively, indicating that ramucirumab provide survival benefit to these type of patients [36]. Another phase III clinical trial (RAINBOW trial) examined the role of the combined use of ramucirumab and paclitaxel in the treatment of patients with advanced gastric cancer after first-line treatment failure. The patients in the ramucirumab group had longer OS than the placebo group (9.6 vs. 7.4 months), and the combined use of ramucirumab and paclitaxel significantly delayed disease progression (PFS 4.4 vs. 2.9 months) [37]. In first-line treatment, some clinical trials have demonstrated that ramucirumab has no significant effects on OS and PFS in terminal patients receiving mFOLFOX6 chemotherapy. Based on the above results, ramucirumab has been approved for patients with gastric cancer who have received an unsuccessful platinum- or fluorouracil-based first-line treatment.

Apatinib

As a small-molecule, targeted drug independently developed by China for the treatment of advanced gastric cancer, apatinib is the only oral drug targeting gastric cancer and is an effective VEGFR2 inhibitor. Phase III clinical trials show that apatinib significantly increases OS and PFS by 55 days (195 days in the apatinib group vs. 140 days in the placebo group) and 28 days (78 days in the apatinib group vs. 53 days in the placebo group), respectively, in patients with advanced gastric cancer who have received an unsuccessful second-line treatment [38].

Multi-target TKI

Sorafenib

Sorafenib is a multi-target TKI that can effectively inhibit the BRAF, VEGF, PDGFR, and Ras/Raf/MERK/ERK pathways. A total of 40 patients were enrolled in the GEMCAD study, in which 2.5% of the patients were evaluated as CR, and 47.2% of the patients were evaluated as SD; grade 3–4 toxic side effects were neutropenia (9.8%), thrombocytopenia (7.3%), neurotoxicity (4.9%), and diarrhea (4.9%); the median PFS and OS were 3 and 6.5 months, respectively; patients with first-line treatment progression time (TTP) > 6 months had a median OS of 9.7 months, whereas patients with a TTP < 6 months had a median OS of 5.6 months. Therefore, TTP in first-line treatment is an effective predictor of prognosis [39]. ECOG5203 studied the role of sorafenib combined with docetaxel and cisplatin in first-line treatment of advanced and metastatic gastric cancer; 44 patients with gastric cancer were included in the trial, with a median PFS and OS of 5.8 and 13.6 months, respectively; the most common toxic side effect was neutropenia; the results of this clinical trial suggest that sorafenib combined with chemotherapy is an effective treatment option in patients with advanced gastric cancer, which needs to be verified with larger clinical studies [40]. Clinical trials about the effect of sorafenib combined with both capecitabine and platinum-based drugs in advanced gastric cancer (NCT00565370) are also in progress.

Sunitinib

Sunitinib is a multi-target TKI that inhibits PDGFR, RET, Flt-3, and VEGFR. A phase II clinical trial of

sunitinib in second-line treatment of advanced gastric cancer showed that median PFS and OS were 2.3 and 6.8 months, respectively; 32.1% of the patients had a SD time not less than 6 weeks; this clinical trial did not reach its primary study endpoint, namely ORR. The above results indicated that sunitinib monotherapy has limited clinical significance in second-line treatment of advanced gastric cancer [41]. In addition, a number of phase I clinical trials explored the safety and efficacy of sunitinib combined with chemotherapy in patients with advanced gastric cancer [42–43].

mTOR inhibitors

The activation of the PI3K/Akt/mTOR signaling pathway is closely related to chemotherapy resistance and prognosis. It has been reported that the mTOR pathway is activated in about 60% of gastric cancer patients, and the activation of this pathway is closely related to the disease progression, thereby making mTOR a potentially effective therapeutic target for gastric cancer [44–45].

Everolimus is an oral mTOR inhibitor that specifically binds its extracellular receptor FKBP12 and exhibits antitumor activity in the treatment of a variety of tumors [46]. A phase I clinical trial demonstrated that capecitabine combined with everolimus showed good tolerability and clinical efficacy in patients with refractory gastric cancer, with a recommended dose of 5 g everolimus administered twice daily [47]. A multicenter everolimus-related phase II clinical trial enrolled 53 patients with refractory metastatic gastric cancer and found that, although no patient achieved CR or PR, the disease control rate reached 56.0%, with a median PFS and OS of 2.7 and 10.1 months, respectively, suggesting that everolimus monotherapy has a very good disease control rate in patients with refractory advanced gastric cancer [48]. The phase III GRANITE study, a clinical trial, showed that everolimus, compared to best support treatment, did not significantly improve median OS (5.4 vs. 4.3 months) and PFS (1.7 vs. 1.4 months) in patients with advanced gastric cancer who showed progression of the disease after first- or second-line treatment [49].

Other targeted drugs

Clinical trials for MET inhibitors, ADP ribose polymerase inhibitors, heat-shock protein 90 inhibitors, and FGFR2 inhibitors are underway to evaluate the efficacy and safety of these drugs in patients with gastric cancer.

Summary

Gastric cancer research has made considerable progress in recent years, but the prognosis of gastric cancer patients has not been significantly improved. Trastuzumab combined with chemotherapy is the standard first-line treatment for HER2-positive advanced gastric cancer, and ramucirumab is approved for second-line treatment. Based on clinical trial data, apatinib may be one of the options for advanced gastric cancer patients who have received unsuccessful first-line chemotherapy. However, whether patients with advanced gastric cancer would benefit from TKI drugs remains yet to be confirmed by more data from phase III clinical trials. Clinical trials report inconsistent results about anti-EGFR drugs in the treatment of gastric cancer, and the main reason may be the difference in enrolled patients among the clinical trials. Everolimus monotherapy achieved good results in phase II clinical trials, but phase III clinical trials have shown that everolimus failed to provide significant survival benefits to patients with advanced gastric cancer. It expected to see increased efficacy of pertuzumab combined with both trastuzumab and chemotherapy in patients with advanced gastric cancer.

Despite the entry of an increasing number of molecularly targeted drugs into clinical trials, the progress achieved in gastric cancer is much less than that in other tumors, which may be accounted for by the following reasons. First, there have been few large-scale molecular genomics studies of gastric cancer until recent years when some large-scale comprehensive molecular typing studies have emerged to provide new ideas for targeted gastric cancer therapy. For example, EBV-positive and MSI patients usually show PIK3CA mutation and CIN patients usually show amplification of tyrosine kinase receptors, which provides a valid basis for the personalized selection of proper targeted therapeutic drugs in the treatment of gastric cancer patients [8]. Second, gastric cancer tumors are a type of highly heterogeneous tumors, showing heterogeneity within a tumor, and heterogeneity between the primary and metastatic tumors—a problem resulting in considerable difficulties for targeted gastric cancer therapy, and presently cannot be solved [50]. Finally, targeted therapy combined with immunotherapy may bring new hope to gastric cancer patients, especially those with MSI-H; however, further investigation in large clinical trials is necessary. With personalized tumor therapy, more preclinical and clinical studies could be expected to enhance prognosis for gastric cancer patients.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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ORIGINAL ARTICLE

Establishment and characterization of an oxaliplatin-resistant hepatic cancer cell line*

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Abstract

Objective The aim of the current study was to establish an oxaliplatin-resistant hepatoma cell line (HepG2/OXA) and investigate the potential mechanisms of its drug resistance.

Methods The hepatoma cell subline, HepG2/OXA, resistant to oxaliplatin (OXA), was established from a parent cell line HepG2, by stepwise exposure to gradually increasing concentrations of OXA over a half-year period. Chemosenstivity of the cytotoxic drugs, OXA, cisplatin (CDDP), adriamycin (ADM), and 5-fuorouracil (5-FU), was determined in HepG2 and HepG2/OXA cells, by the Cell counting kit-8 (CCK8) assay. Cell cycle distribution of HepG2 and HepG2/OXA cells was analyzed by Flow cytometry (FCM). The expression levels of several drug resistance-related proteins, such as P-glycoprotein (P-gp), multidrug resistant protein 1 (MRP1), and excision repair-cross complementing 1 (ERCC1) protein in the two cell lines were tested by the western blot assay.

Results The IC50 of OXA in HepG2/OXA and HepG2 were 136.84 μmol/L and 23.86 μmol/L, respectively. The resistance index (RI) was 5.34. HepG2 was also demonstrated to be cross-resistant to other anti-tumor agents, such as 5-FU, ADM, and CDDP. The percentage of HepG2/OXA cells in the S phase was significantly decreased compared to HepG2 cells (25.58% ± 2.36% vs 14.37% ± 2.54%, $P < 0.05$), while the percentage of cells in the G0/G1 and G2/M phases showed no statistical difference (respectively 55.29% ± 4.98% vs 56.73% ± 4.56%, $P > 0.05$, and 24.63% ± 4.81% vs 28.26% ± 3.82%, $P > 0.05$). The ERCC1 was found to be over expressed in HepG2/OXA cells, while there was no difference in the expressions of P-gp and MRP1 between the multiple drug resistance (MDR) phenotype cell line and its parental cell line.

Conclusion HepG2/OXA showed an MDR ability; the over expression of ERCC1 might be associated with the platinum resistance of the cells, but P-gp and MRP1 are not.

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Key words: hepatocellular carcinoma (HCC); multidrug resistance (MDR); excision repair-cross complementing 1 (ERCC1); oxaliplatin

In China, hepatocellular carcinoma (HCC) is the fourth most common malignancy in men and the eighth in women, and the fourth most common cause of cancer-related mortality in men and women [1]. Chronic HBV infection is thought to be the most important risk factor for the pathogenesis of HCC, especially in China. Unfortunately, the majority of the patients are surgically unresectable at the time of diagnosis, and the risk of recurrence for those postoperative cases is considerably high. As a result, even though the diagnosis and comprehensive treatments are improved, the prognosis of HCC remains extremely poor [2].

Due to the lack of powerful research data from evidence-based medicine, the effect of chemotherapy on the overall survival remains to be controversial. However, chemotherapy is still an important strategy for most

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unresectable and recurrent cases. Platinum drugs, such as cisplatin (DDP) and oxaliplatin (OXA) have been used as the common chemotherapeutic agents in the treatments for HCC. The international multi-center randomized phase III study (EACH) confirmed the therapeutic value of FOLFOX4 (OXA, CF plus 5-FU) for advanced HCC, and it was the first time that a combined regimen of oxaliplatin was proved to have survival benefits for patients with advanced HCC [3]. However, the effect of oxaliplatin is limited due to the intrinsic or acquired drug resistance of HCC cells [4–5]. Additionally, the potential mechanism of OXA resistance remains unclear.

OXA resistance is often accompanied by cross-resistance to other chemotherapy agents, called multidrug resistance (MDR), which is thought to be the critical reason for the failure of chemotherapy treatment [6]. One of the possible mechanisms of MDR is the drug efflux pumps which lead to the decrease of the intracellular concentration of the chemotherapeutic agents. The drug pumps mainly include ATP-binding cassette (ABC) transporters, such as P-gp, MRP1, and lung resistant-related protein (LRP). However, since the cytotoxicity of platinum is mediated by DNA damage, nucleotide excision repair (NER) plays a critical role in the occurrence of platinum-related resistance [7–8]. ERCC1 over-expression enhances the rapid DNA damage repair ability and is an important factor for platinum resistance [9].

In the current study, we established an OXA-resistant cell line, HepG2/OXA, from its parental cell line HepG2, analyzed its biological characteristics, and explored its potential molecular mechanism of MDR *in vitro*.

Materials and methods

Compounds

Chemotherapeutic drugs, oxaliplatin (OXA; St. Louis, MO, USA), 5-fluorouracil (5-FU; Jiangsu Hen Rui Medicine Co. Ltd, Lianyungang, China), cisplatin (CDDP; Jiangsu Hen Rui Medicine Co. Ltd, Lianyungang, China), doxorubicin (ADM; Jiangsu Hen Rui Medicine Co. Ltd, Lianyungang, China) were used in this study. All the agents were used according to the protocol provided by the manufacturers.

Cell lines and culture conditions

Two human hepatoma cell lines (HepG2 and SMMC-7721) were used in the present study. HepG2 and SMMC-7721 cells were high-malignancy hepatocarcinoma cell lines established from surgically excised specimens of Chinese hepatocellular carcinoma patients, and derived from the China Center for Type Culture Collection. HepG2 and SMMC-7721 cells were cultured in Dulbecco's modified Eagle's medium/high glucose (DMEM/H, Invitrogen Carlsbad, CA, USA) supplemented with 10% (v/v) fetal bovine serum (Invitrogen Carlsbad, CA, USA), 200 IU/mL penicillin (ICN Biomedical, Costa Mesa, CA, USA), 100 mg/mL streptomycin (ICN Biomedical), and 0.5 mmol/L sodium pyruvate (Cambrex, Walkersville, MD, USA). The cells were cultured at 37 °C in a humidified atmosphere, under conditions of 5% CO_2.

Establishment of OXA-resistant subline *in vitro*

The parental cell lines HepG2 and SMMC-7721 were exposed to medium containing OXA at an initial concentration of 25 μM for 24 h. The dead cells were removed with 0.01 mol/L phosphate-buffered saline (PBS), and the remaining cells were cultured in OXA-free culture medium. Once the growth of treated cells reached 70%–80% confluence, the above protocol was repeated. After half a year, a resistant sub-clone of HepG2/OXA was obtained, which could grow exponentially in the medium containing 10 μM OXA. The proliferation of the parental cell line SMMC-7721 was poor; the OXA-resistant sub-line could not be achieved even when the culturing time was prolonged to one year.

Proliferation assay after co-exposure to chemotherapeutic drugs

The proliferation rate of cells under the conditions of exposure to the chemotherapeutic drugs was determined by the CCK-8 assay (Dojindo Molecular Technology, Dojindo, Japan), according to the manufacturer's instructions. We compared the proliferation rates at different concentrations of the drugs to clarify the characteristics of MDR in HepG2/OXA cells. Before the assay, HepG2/OXA cells were maintained in OXA-free medium and cultured for three generations. Cancer cells were seeded into two 96-well plates at a concentration of 8000 cells/well with 100 μL of medium and cultured for 12 h. Then, experiment groups (EG) were cultured in 100 μL of fresh medium containing various concentrations of the anticancer drugs for 24 h, while the control group (CG) was cultured in 100 μL of medium free of anticancer drugs. The concentrations of OXA were 3.125, 6.25, 12.5, 25, 50, and 100 μM; those of CDDP were 2.5, 5, 10, 20, 40, and 80 μM; those of 5-FU were 12.5, 25, 50, 100, 200, and 400 μg/mL; and those of ADM were 1, 2, 4, 8, 16, and 32 μM. The medium was discarded after 24 h, and cells were incubated for 2 h in 110 μL of DMEM/H solution with CCK-8 (10 μL of CCK-8 and 100 μL of DMEM/H). Blank groups (BG) were made in wells without cells. Optical density (OD) was measured for each well at a wavelength of 450 nm. Inhibitory ratio (IR) in different concentrations was calculated by the following formula: $1 - (OD_{EG} - OD_{BG}) / (OD_{CG} - OD_{BG})$. According to the IRs in different concentrations of the anticancer drugs, the 50% inhibitory dose (IC50) was measured by SPSS 19.0 (SPSS Inc, Chicago, IL, USA). Resistant indexes ($RI = IC50_{HepG2/}$

Table 1 Determination of IC50 and resistance index (RI) according to the inhibition ratio of different concentrations of OXA of HepG2/OXA cells and HepG2 cells (mean ± SD; n = 5)

	Inhibition ratio of different concentrations of OXA (μmol/L)						IC50	RI
	3.125 μM	6.25 μM	12.5 μM	25 μM	50 μM	100 μM		
HepG2/OXA	10.18 ± 1.81	12.49 ± 2.68	13.01 ± 2.07	13.92 ± 3.43	26.36 ± 6.27	63.32 ± 7.26	136.84 ± 8.46	5.74
HepG2	12.89 ± 1.76*	16.59 ± 2.97*	21.14 ± 2.29*	28.13 ± 4.15**	51.31 ± 6.89**	96.68 ± 8.24**	23.86 ± 3.78	

* P < 0.05, ** P < 0.01

$_{OXA}$ / $IC50_{HepG2}$) of each tested drug were calculated. Five replicate wells were used for each drug concentration and the testing was carried out independently for three times.

Cell cycle analysis

The HepG2/OXA cells and the parental HepG2 cells were harvested after digestion by trypsinogen (Invitrogen, Carlsbad, CA, USA) without ethylene diamine tetraacetic acid (Invitrogen, Carlsbad, CA, USA), washed two times with ice-cold PBS (pH 7.2), and immobilized using dehydrated alcohol (–20 °C) and PBS (3:1) at 4 °C for 12 h. The immobilized cells were then washed twice with ice-cold PBS and incubated in the presence of 25 mg/mL RNase (Invitrogen, Carlsbad, CA, USA) at 37 °C for 30 min, stained with 50 mg/mL PI (Invitrogen, Carlsbad, CA, USA), and then placed on ice for 30 min in the dark. Cell cycle phase distribution was analyzed using a flow cytometer (Becton Dickinson, Biosciences, San Jose, CA, USA). Data from 10 000 cells were collected and analyzed using the Modofit software program (Becton Dickinson, Biosciences, San Jose, CA, USA).

Expression of P-gp, MRP1, ERCC1 by western blotting

Total protein was collected from the cultured HepG2/OXA cells and the parental HepG2 cells. The protein concentration was measured by a BCA Protein Assay Kit (Beyotime Institute of Biotechnology, Jiangsu, China). Before electrophoresis, the protein was denatured in lithium dodecyl sulfate (LDS) sample buffer (106 mmol/L Tris-HCl, 141 mmol/L Tris base; pH 8.5, 0.51 mmol/L EDTA, 10% glycerol, 2% LDS, 0.22 mmol/L SERVA blue G250, 0.175 mmol/L, phenol red, 0.1 mmol/L 2-mercaptoethanol) for 10 min at 95 °C. Total protein (20 μg per lane) was electrophoresed on an 8% SDS-PAGE gel and transferred onto a 0.45-μm nitrocellulose filter membrane (NC) (Roche, Indianapolis, IN, USA). Membranes were blocked with 5% (w/v) nonfat dry milk in PBST (phosphate-buffered saline containing 0.05% Tween 20) for 2 h at room temperature and incubated overnight at 4 °C with antibodies against P-gp (1:200), or MRP1 (1:200), or ERCC1 (1:100) (Santa Cruz, CA, USA). The membranes were then incubated with Dylight™ 800-Labeled antibodies (Gaithersburg Biotechnology, MD, USA) for 1 h after being washed four times with PBST for 5 min. In the end, the immunoblot signals were scanned and analyzed by the Odyssey Infrared Imaging System (Li-Cor Biosciences Nebraska USA).

Table 2 Results of IC50 and resistance index (RI) of different anticancer drugs of HepG2/OXA cells and HepG2 cells (mean ± SD)

Drugs	IC50 (n = 5)		RIs
	HepG2 (μmol/L)	HepG2/OXA (μmol/L)	
OXA	23.86 ± 3.78	136.84 ± 8.46**	5.74
DDP	7.56 ± 1.71	12.33 ± 2.29*	1.63
5-FU	109.84 ± 6.84	154.01 ± 8.93*	1.40
ADM	4.45 ± 1.12	8.67 ± 1.97*	1.94

* P < 0.05, ** P < 0.01

Statistical analysis

All digital results were displayed as mean ± SD. The quantitative ratios of different groups were compared by Student's t-test with SPSS 13.0. Probability values of P < 0.05 were regarded as statistically significant. All statistical tests were two-sided. All the experiments were repeated at least three times.

Results

Establishment of the OXA-resistant cell line HepG2/OXA and determination of its MDR

The HepG2/OXA cell line was established by pulsed exposure to a high concentration of OXA. The cytotoxicity assay (CCK8) showed that HepG2/OXA cells were resistant not only to OXA, but also to other anticancer drugs. The IC50 of HepG2/OXA for OXA was (136.84 ± 8.46) μmol/L, which was 5.74 times that of the parental cells HepG2 [(23.86 ± 3.78) μmol/L]. In addition, the RIs of HepG2/OXA cells were 1.63, 1.40, and 1.93 for DDP, 5-FU, and ADM, respectively (Tables 1 and 2).

Cell cycle distribution of HepG2/OXA and HepG2

The cell cycle distribution in the HepG2/OXA cell line exhibited a significantly decreased percentage of cells in the S phase (25.58% ± 2.36% vs 14.37% ± 2.54%, P < 0.05) in comparison with the HepG2 cells, while the percentage of cells in the G0/G1 phase (55.29% ± 4.98% vs 56.73% ± 4.56%, P > 0.05) and G2/M phase (24.63% ± 4.81% vs 28.26% ± 3.82%, P > 0.05) showed no statistical differences (Fig. 1).

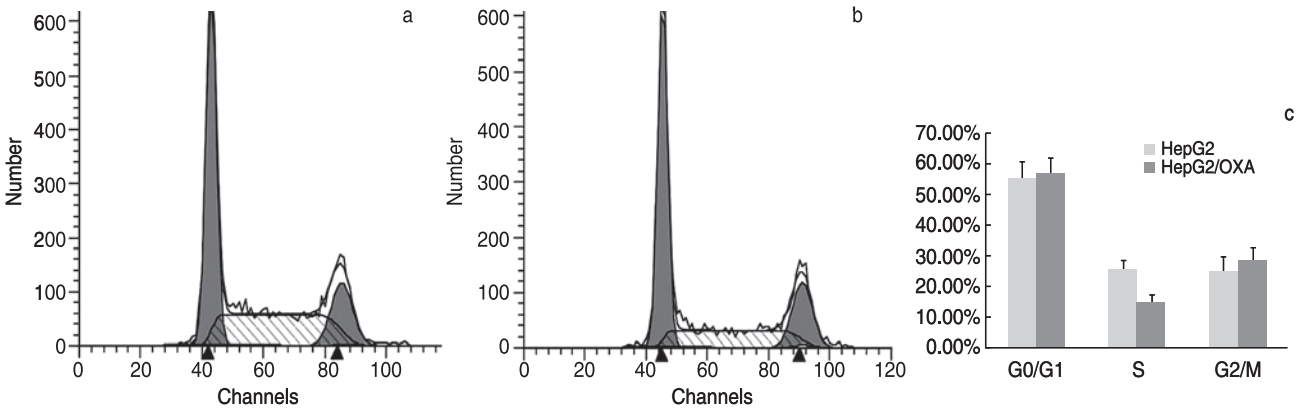

Fig. 1 (a) Cell cycle distribution of HepG2 cells; (b) Cell cycle distribution of HepG2/OXA cells; (c) The percentage of G0/G1, S, G2/M cells of HepG2 and HepG2/OXA

The distribution of HepG2/OXA cells in the S phase exhibited a significant decrease, in comparison with HepG2 cells, while the percentages of the cells in the G0/G1 and G2/M phases showed no statistical differences.

ERCC1, P-gp, and MRP1 protein expression in HepG2/OXA and HepG2 cells

P-glycoprotein (P-gp), multidrug-resistant protein 1 (MRP1), and ERCC1 protein expression in HepG2/OXA cells compared with that in the parental cells was determined by western blotting. ERCC1 was overexpressed in HepG2/OXA cells in comparison with HepG2 cells. The ratio of ERCC1 and β-actin grey intensity was 0.099 ± 0.014 and 0.396 ± 0.040, respectively, in the HepG2 and HepG2/OXA cells; the difference was statistically significant ($P < 0.0001$). However, the protein expression levels of P-gp (0.788 ± 0.085 vs 0.740 ± 0.063, $P = 0.367$) and MRP1 (0.374 ± 0.060 vs 0.350 ± 0.073, $P = 0.468$) between the MDR phenotype cells and the parental cells showed no significant difference. Data is shown in Table 3 and Fig. 2.

ERCC1 was over-expressed in the HepG2/OXA cells. The ratio difference of ERCC1 and β-actin grey intensity in HepG2/OXA and HepG2 was significant ($P < 0.0001$). However, there was no significant difference between the P-gp and MRP1 protein expression levels in the MDR phenotype cells and the parental cells.

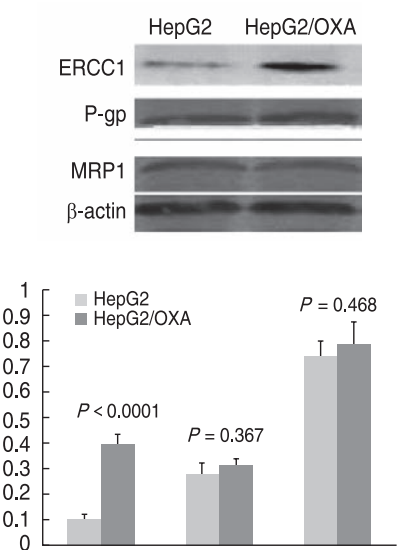

Fig. 2 ERCC1, P-gp, and MRP1 protein expression levels in HepG2/OXA and HepG2 cells were assayed by western blot

Table 3 ERCC1, P-gp, and MRP1 protein expression levels in HepG2/OXA and HepG2 cells (mean ± SD, $n = 5$)

Cell lines	ERCC1	P-gp	MRP1
HepG2	0.099 ± 0.013	0.740 ± 0.063	0.350 ± 0.073
HepG2/OXA	0.396 ± 0.040**	0.788 ± 0.085	0.374 ± 0.060

** $P < 0.01$

Discussion

The effect of chemotherapy on the overall survival of most HCC patients has been constantly controversial. However, a randomized phase III trial (EACH) presented in the 2010 ASCO meeting indicated that FOLFOX4 significantly improved the median progression-free survival from 1.77 to 2.93 months ($P = 0.0002$), and overall survival from 4.90 to 6.47 months ($P = 0.00425$). Response rates (complete and partial responses), as calculated by RECIST (Response Evaluation Criteria in Solid Tumors) were 8.7% for FOLFOX4 and 2.7% for doxorubicin ($P = 0.0142$). Disease control rates (complete and partial responses and stable disease) were 53% for FOLFOX4 vs 32% for doxorubicin ($P < 0.0001$). It is the first survival advantage report for HCC for any chemoregimen [10]. Drug resistance is the main reason for the failure of chemotherapy in the treatment of malignant tumors [11]. Undoubtedly, reducing or reversing the drug resistance

may improve the benefits of chemotherapy and overall survival. As OXA is one of the most useful drugs for the treatment of malignant tumors including HCC, which has a different mechanism of drug resistance than CDDP [12], it is necessary to elucidate the mechanism of OXA resistance.

Once malignant tumor cells gain resistance against one drug, they may be resistant to other chemotherapy drugs as well [13]. In this study, an OXA-resistant hepatoma cell line (HepG2/OXA) was established by discontinuously exposing the cell line to high concentrations of OXA for half a year. The results of the CCK8 assay showed that the IC50 of the HepG2/OXA cells was 5.34 times more than that of the HepG2 cells. The OXA-resistant cells also developed cross-resistance to other anti-tumor agents like 5-FU, ADM, and CDDP. The growth rate of HepG2/OXA cells was lower than that of the parental cells. This result is similar to that of a study on a CDDP-resistant hepatoma cell line SK-Hep-1/CDDP, which was established by Zhou *et al* [14]. As far as the cell cycle distribution of the HepG2/OXA cell line was concerned, there was a difference between HepG2/OXA and SK-Hep-1/CDDP cell lines. There was a significantly decreased percentage of HepG2/OXA cells in the S phase, when compared to the case for HepG2 cells. Conversely, the proportion of SK-Hep-1/CDDP cells in both the G2/M and S phases increased significantly, when compared with the parental cells. The reason for this dissimilarity might be the difference between the drugs and cell lines.

The relative mechanism of the phenomenon of multidrug resistance is considered to be related with drug efflux pumps mediated by ATP-binding cassette (ABC) transporters such as P-gp, MRP1, and lung resistant-related protein (LRP) [15]. Inhibiting MDR transporter function can reverse the MDR phenotype and improve the sensitivity of chemotherapy [16–17]. In case of HCC, some MDR cell lines like HepG2/ADM [18] and SMMC7721/ADM [19] overexpressed the ABC protein, compared to their parental cell lines. Nevertheless, in our experiment, neither P-gp nor MRP1 expression was significantly increased in HepG2/OXA cells, when compared to the case for HepG2 cells. However, ERCC1, one of the rate-limiting enzymes in NER, was observed to be overexpressed in the MDR phenotype cells. This demonstrated that there was another mechanism for OXA resistance in HepG2/OXA cells, besides the decrease of drug concentration in the cytoplasm due to the overexpression of ABC [20]. It is well-known that more than 85% of HCC cases are accompanied with cirrhosis [21]. Increased DNA repair activity in cirrhosis with inflammatory activity may reflect increased DNA damage as a consequence of chronic liver injury [22]. Meanwhile, ERCC1, which is involved in the early steps of the NER process, is associated with liver fibrogenesis and cancer, and it could be related to the well-recognized resistance of HCC to chemotherapeutics [23]. In addition, increased ERCC1 expression is associated with CDDP resistance in HCC specimens and cell lines. Immunohistochemical analysis for resected HCC tissues may be a useful predictor for the effectiveness of adjuvant chemotherapy using CDDP [24–25]. Therefore, ERCC1 overexpression plays an extremely important role in HCC chemotherapeutic drug resistance, especially for platinum resistance.

In summary, although a chemotherapeutic regimen including OXA was proved to have survival benefits for HCC, the need to establish drug resistance models, especially for platinum resistance, is urgent, so as to elucidate its mechanism and surmount drug resistance. An OXA-resistant human hepatoma cell line, HepG2/OXA, which was established by discontinuously exposing the parental cell line, HepG2, to a high dose of OXA, provided a stable cell model for the further study of platinum resistant mechanisms and the reversal of clinical HCC drug resistance.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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ORIGINAL ARTICLE

Nutritional status changes in patients with advanced non-small cell lung cancer receiving first-line chemotherapy

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Abstract

Objective This study aimed to assess the real-life nutritional status changes and gastrointestinal symptoms in patients with advanced non-small cell lung cancer (NSCLC) receiving chemotherapy.

Methods A total of 104 patients with metastatic NSCLC receiving first-line chemotherapy were included in this study. Unintentional weight loss, body mass index (BMI) changes, and gastrointestinal symptoms were recorded and evaluated. Biochemical parameters [hemoglobin (Hb) and albumin levels] were compared before and after two chemotherapy cycles using SPSS software.

Results Of these patients, 65.38% (68/104) experienced unintentional weight loss, whereas 30.77% and 12.5% presented with ≥ 5% and ≥ 10% degrees of weight loss, respectively, within 6 months before first-line chemotherapy was administered. Then, 48.08% (50/104) of the patients experienced unintentional weight loss after two chemotherapy cycles. The mean body weight after chemotherapy was 61.47 ± 10.37 kg, which was significantly decreased relative to that before chemotherapy ($P < 0.05$). The mean BMI after chemotherapy was 22.66 ± 3.34 kg/m^2, which was also significantly diminished with respect to that during the previous chemotherapy cycle ($P < 0.05$). The most common gastrointestinal symptoms reported among all the study patients were anorexia (80/104, 76.92%), nausea (53/104, 50.96%), constipation (49/104, 47.12%), vomiting (48/104, 46.15%), taste disorders (40/104, 38.46%), and early satiety (32/104, 30.77%). The mean Hb levels after chemotherapy were 117.06 ± 16.67 g/L, which were significantly lower than those before chemotherapy (132.73 ± 16.42 g/L) ($P < 0.05$). No significant difference was noted between the mean albumin levels before and after chemotherapy (38.29 ± 4.22 g/L vs 38.17 ± 4.54 g/L; $P = 0.798$).

Conclusion Weight loss history, gastrointestinal symptoms, and Hb level decreases are determinant factors of nutritional status in patients with advanced NSCLC and must be included in the screening, evaluation, and treatment of lung carcinoma.

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Keywords: lung cancer; gastrointestinal symptoms; weight loss; chemotherapy; hemoglobin; albumin

Non-small cell lung cancer (NSCLC) is the most common cancer and the major cause of cancer-related deaths in China and globally [1]. Systemic chemotherapy is the mainstream treatment for metastatic NSCLC without a driving gene, with an objective tumor response rate of 25–35% [2]. At cancer diagnosis, approximately 50% of patients present with some nutritional deficits [3]. This prevalence may even rise depending on the tumor location and stage. The highest prevalence is noted in patients with tumors of the gastrointestinal tract and the lungs [4].

Systemic administration of chemotherapy agents targets rapidly dividing cells, including those in the bone marrow and gastrointestinal tract epithelial lining. These direct effects of chemotherapy agents can result in gastrointestinal toxicities, which in turn affect the nutritional statuses of patients [5]. Chemotherapy-induced nausea, vomiting, diarrhea, constipation, anorexia, taste disorder, and early satiety are the symptoms commonly

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reported by patients undergoing chemotherapy [6]. A subset of these patients may experience the symptoms to an extent that limits their dietary intake, and their nutritional statuses may be compromised, leading to negative outcomes for patients and treatment facilities [7]. Malnourished patients experience decreased quality of life, diminished treatment tolerance, increased number of complications, and prolonged hospital admissions, all of which jeopardize treatment adherence and tumor control and ultimately increase the mortality and healthcare burden [8]. Therefore, detecting malnutrition early in patients with cancer has become increasingly important.

Nutritional screening includes anthropometric parameters [body mass index (BMI) and weight loss percentage] and biochemical parameters [hemoglobin (Hb) and albumin] [9-12]. Gastrointestinal symptoms, weight loss, and Hb and albumin levels often decrease in patients receiving chemotherapy [13]. An easy routine screening of malnutrition in patients with cancer should include these factors.

The current study aimed to assess the real-life nutritional status changes and gastrointestinal symptoms in patients with advanced NSCLC receiving chemotherapy.

Materials and methods

This cross-sectional study was conducted at the Cancer Centre, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, between January 2016 and January 2017. Informed consent was obtained from all participants. Biochemical and clinical data were assessed before the first chemotherapy cycle and after the second chemotherapy cycle.

The selection criteria were as follows: age between 19 and 75 years and pathological diagnosis of stage IV NSCLC. Meanwhile, patients who underwent any surgery or radiotherapy as treatment were excluded from the study. The present research was conducted in accordance with the guidelines in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the ethics committee.

Data analysis

Descriptive statistics were used for the qualitative and quantitative variables, frequency, percentage, mean, and standard deviation (SD). The average distance of the groups was compared using the paired-samples *t* test. Data analysis was performed using the SPSS software, version 18 (SPSS, Inc., USA). $P < 0.05$ was considered statistically significant.

Table 1 General characteristics (mean values and standard deviations; number of patients and percentage values)

Variables	*n*		%
Sex			
Male	70		67.31
Femal	34		32.69
Age (years)			
Mean		54.57	
SD		9.32	
Weight (kg)			
Mean		62.28	
SD		10.05	
BMI (kg/m^2)			
Mean		22.98	
SD		3.19	
Weight loss (kg)			
Mean		2.38	
SD		2.85	
Weight loss			
≥ 5	32		30.77
≥ 10	13		12.50

Results

General characteristics

A total of 104 patients with advanced NSCLC [34 (32.69%) women and 70 (67.31%) men] underwent at least two chemotherapy cycles. The mean age was 54.57 (SD 14.8) years. The mean body weight was 62.28 ± 10.46 kg, and the mean BMI was 22.98 ± 3.19 kg/m^2 (Table 1).

Unintentional weight loss before chemotherapy

Over 65.38% (68/104) of the patients experienced unintentional weight loss, whereas 30.77% and 12.50% showed ≥ 5% and ≥ 10% degrees of weight loss, respectively, within 6 months before first-line chemotherapy was administered (Table 1). Unintentional weight loss > 10% in the preceding 6 months was considered a sign of malnutrition.

Gastrointestinal symptoms during chemotherapy

The most common gastrointestinal symptoms reported among all the study patients were anorexia (80/104, 76.92%), nausea (53/104, 50.96%), constipation (49/104, 47.12%), vomiting (48/104, 46.15%), taste disorders (40/104, 38.46%), early satiety (32/104, 30.77%), diarrhea (13/104, 12.50%), and dysphagia (2/104, 1.92%; Table 2).

Table 2 Gastrointestinal symptoms reported during chemotherapy

Symptoms	*n*	%
Anorexia	80	76.92
Nausea	53	50.96
Constipation	49	47.12
Vomiting	48	46.15
Taste disorders	40	38.46
Early satiety	32	30.77
Diarrhoea	13	12.50
Dysphagia	2	1.92

Weight loss and BMI changes after chemotherapy

Of all patients, 48.08% (50/104) experienced unintentional weight loss after two chemotherapy cycles. The mean body weight after chemotherapy was 61.47 ± 10.37 kg, which was significantly decreased relative to that before chemotherapy ($P < 0.05$). The mean BMI after chemotherapy was 22.66 ± 3.34 kg/m^2, which was also significantly decreased relative to that during the previous chemotherapy cycle ($P < 0.05$) (Table 3).

Hb and albumin level changes after chemotherapy

The mean Hb levels before chemotherapy were 132.73 ± 16.42 g/L, which were significantly decreased relative to those after chemotherapy (117.06 ± 16.67 g/L) ($P < 0.05$). No significant difference was noted between the mean albumin levels before and after chemotherapy (38.29 ± 4.22 g/L vs 38.17 ± 4.54 g/L; P=0.798) (Table 3).

Discussion

Malnutrition affects 20–70% of patients with cancer [14]. Weight loss is an easy measure of diagnosing malnutrition and should be assessed in daily practice. However, slight changes in nutritional status can be overlooked occasionally. Declining nutritional status and weight loss originate from multiple processes and are associated with decreased responses to chemotherapy treatment and reduced survival [15]. Therefore, all patients with cancer must be evaluated for early signs of malnutrition and weight loss to provide adequate nutritional support and improve the quality of life and treatment response in these patients. Clinicians and patients must be aware of the effects of malnutrition on patient outcomes, particularly those in patients receiving chemotherapy. Changes in nutritional status have been associated with altered absorption, metabolism, and elimination of chemotherapy drugs. The prevalence of unintentional weight loss in patients with NSCLC has been reported to be 38% [16]. Moreover, most patients with advanced NSCLC also present with malnourishment [17–19]. The present study observed similar results, and 65.38% (68/104) of the patients experienced unintentional weight loss, whereas 30.77% and 12.5% of the patients manifested ≥ 5% and ≥ 10% degrees of weight loss, respectively, within 6 months before first-line chemotherapy was administered. The etiology of unintentional weight loss is not well understood and may be due to decreased food intake.

Table 3 Weight, BMI and biochemical parameters change during chemotherapy (Mean ± SD)

Variables	Before chemotherapy	After chemotherapy	*P*
Weight (kg)	62.28 ± 10.46	61.47 ± 10.37	0.001
BMI (kg/m^2)	22.98 ± 3.19	22.66 ± 3.34	0.004
Hb (g/L)	132.73 ± 16.42	117.06 ± 16.67	0.000
Albumin (g/L)	38.29 ± 4.22	38.17 ± 4.54	0.798

BMI is another very important nutritional index and the most practical and simplest means to assess nutritional status. However, this measure provides little information on the body composition alteration in cachexia [20]. In the present study, the mean BMI of the patients was 22.98 kg/m^2.

Gastrointestinal symptoms are noteworthy components of malnutrition in patients with cancer. Upper gastrointestinal symptoms reported by patients are important because a high prevalence of these symptoms can cause difficulty in feeding, reduction in energy intake, and worsening of nutritional status [21–23]. In the current study, the most frequent gastrointestinal symptoms were anorexia (80/104, 76.92%), nausea (53/104, 50.96%), constipation (49/104, 47.12%), vomiting (48/104, 46.15%), taste disorders (40/104, 38.46%), early satiety (32/104, 30.77%), diarrhea (13/104, 12.50%), and dysphagia (2/104, 1.92%). A similar prevalence of gastrointestinal symptoms was found in other studies [18–20].

Consistent with other reports, the Hb levels were significantly decreased after chemotherapy in our study. This result may be due to the toxic effects of the chemotherapeutic drugs on hematopoietic cells and gut epithelia that lead to malabsorption [24].

Serum albumin is the simplest and most effective variable indicating visceral protein function. Therefore, this biomarker is commonly used in assessing malnutrition. Normal serum albumin levels range between 3.5 and 5.0 g/dL in adults. Hypoalbuminemia is defined as serum albumin levels < 3.5 g/dL. Albumin is habitually included among the parameters utilized for nutritional assessment and has recently become further widespread. Serum albumin concentration has also been established as an independent prognostic variable

for survival in advanced NSCLC [25]. Nevertheless, scarce data are available to date on the prevalence and clinical significance of hypoalbuminemia in patients with cancer and how such conditions affect cancer treatment. In the present study, the albumin levels did not diminish after chemotherapy, unlike in other studies. The discrepancy may be due to the small sample size and short investigation time in the current work.

In this study, patients with advanced lung cancer showed a high prevalence of weight loss. Gastrointestinal symptoms, such as anorexia (80/104, 76.92%), nausea (53/104, 50.96%), constipation (49/104, 47.12%), vomiting (48/104, 46.15%), taste disorders (40/104, 38.46%), and early satiety (32/104, 30.77%), were very common during chemotherapy. Chemotherapy can induce weight loss and Hb level decreases in patients with advanced lung cancer.

In conclusion, weight loss history, gastrointestinal symptoms, and Hb level decreases are determinant factors of nutritional status in patients with advanced lung cancer and must be included in the screening, evaluation, and treatment of lung carcinoma.

Conflict of interest

The authors indicated no potential conflicts of interest.

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ORIGINAL ARTICLE

Prognostic significance of the number of pelvic lymph nodes removed in patients with early cervical cancer

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Abstract

Objective The aim of this research was to study the prognostic significance of the number of pelvic lymph nodes removed in patients with early cervical cancer.

Methods We searched the PubMed database using the terms "cervical cancer" and "lymph nodes" or "lymphadenectomy". Studies on the association between number of lymph nodes removed and prognosis or survival were identified. We retrospectively studied the relevant research.

Results Ten retrospective studies were included. Two studies indicated that the number of lymph nodes had no association with prognosis whereas three studies found a positive relationship. Five studies indicated some factors that could influence the relationship between number of lymph nodes and prognosis.

Conclusion The number of lymph nodes removed may positively influence the prognosis of patients with cervical cancer. Some factors may influence the relationship between the extent of lymph nodes removed and patient prognosis. Additional multicenter, prospective studies with large samples are required to confirm the study findings.

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Key words: cervical cancer; prognosis; number of lymph nodes; pelvic lymphadenectomy

History and background

Although pelvic lymphadenectomy has been used traditionally for more than a century in the surgical treatment of early cervical cancer, uniform conclusions have not been reached regarding the optimal number of lymph nodes that should be removed within the standard scope of lymphadenectomy. Standardizing lymphadenectomy is essential for further improvement of surgical quality and for improved survival of patients with cervical cancer. We reviewed existing studies on the prognostic value of the number of lymph nodes retrieved.

Tracing the development history of cervical cancer surgical treatment, Ernst Wertheim standardized radical abdominal hysterectomy in 1912, which made this cancer curable and formed the basis of current treatment for early-stage cervical cancer. Dr. Fred J. Taussig subsequently realized the importance of careful removal of the pelvic nodes during this operative procedure. Dr. Taussig believed that even if metastatic disease had been eradicated from the lymph nodes, recurrence might take place in the pelvic nodes. Therefore, he dissected these lymph nodes and their lymphatic channels en bloc during surgery [1]. After that, Joe Vincent Meigs combined radical abdominal hysterectomy with Taussig's en bloc pelvic lymph node dissection procedure, establishing a milestone in the treatment of cervical cancer. From his vast experience, Dr. Meigs felt that positive lymph nodes could not be detected by inspection, palpation, or visualization and that the only way to determine whether lymph nodes were positive was via pathological examination in the laboratory after their removal [1]. Thereafter, lymph node dissection was performed together with radical hysterectomy, achieving an 89.7% 5-year survival rate for stage I disease, and a 63.0% 5-year survival rate for stage II disease, far surpassing Wertheim's 18.4% overall 5-year survival rate [1]. Meigs demonstrated that lymphatic invasion was much more common than previously believed and that en bloc resection of lymphatic tissue afforded greater survival benefit. As a result of these efforts, pelvic lymphadenectomy has been performed for years.

Today, cervical cancer remains the fourth most prevalent female malignancy and the fourth leading cause of cancer deaths in women worldwide [2]. With

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gradual popularization of cervical cancer screening, the early diagnosis rate of cervical cancer has improved, and the proportion of detection of early cervical cancer has increased. For early cervical cancer, the present standard method of surgical treatment is radical hysterectomy plus pelvic lymph node dissection, with or without para-aortic lymph node sampling; systematic pelvic lymph node dissection is an integral part of the surgical procedure recommended to treat early-stage cervical cancer. However, no consensus has been reached as to what extent pelvic lymphadenectomy should be conducted, and there are no unified conclusions about the scope of pelvic lymphadenectomy in cervical cancer.

Benedetti-Panici *et al* found that in patients with cervical cancer, the lymph node metastasis rate was 7% in the deep common iliac, 28% in the superficial common iliac, 7% in the deep obturator, 86% in the superficial obturator, 29% in the external iliac, 8% in the internal iliac, 7% in the presacral, and 29% in the parametrial lymph nodes [3]. Therefore, for the purpose of treatment, those researchers suggested that systematic dissection of all the lymphatic tissue located around the cervix and the pelvic vessels should be performed in these patients, to completely remove potential sites of metastasis, including the eight groups of lymph nodes listed above [3]. In reports by Lim *et al* and by Pieterse *et al*, pelvic lymphadenectomy included the bilateral common iliac, external iliac, internal iliac, and obturator, a total of four groups of lymph nodes [4–5]. However, Ditto *et al* included the presacral lymph nodes in addition to these four groups [6]. Subsequently, Batista *et al* pointed out that systemic lymphadenectomy also included the parametrial lymph nodes in addition to the above four groups of lymph nodes [7]. Hu *et al* felt that systematic pelvic lymphadenectomy should also include the inguinal lymph nodes in addition to the above four groups [8]. The scope of lymphadenectomy can directly influence the number of pelvic lymph nodes dissected (NPLD). Moreover, the NPLD can vary greatly depending on the anatomy of the patient, the status of lymphoid tissue peripheral inflammation and adhesion, the standards of the surgeon, the extent of surgery, and examination by a pathologist. In previous studies, the mean or median number of pelvic nodes removed ranged from 13–65 [9–10]. In a report by Verleye *et al*, removal of more than 11 pelvic nodes was suggested as one of the quality indicators for pelvic lymphadenectomy [11]. Up to now, how many lymph nodes should be removed to obtain the best treatment effect remains unknown. Some studies have focused on the relationship between the prognosis of patients with pelvic lymphadenectomy and the number of lymph nodes removed, with the aim of helping to standardize pelvic lymphadenectomy in early cervical cancer.

Related research

Whereas some studies proved a positive correlation between the number of lymph nodes removed and the prognosis of patients with cervical cancer, no obvious correlation was found in other studies. Suprasert *et al* retrospectively analyzed 826 patients with radical hysterectomy and pelvic lymphadenectomy, stratifying them into four groups according to NPLD: 11–20, 21–30, 31–40, and ≥ 41 lymph nodes. The researchers found no statistically significant difference among the four groups with respect to 5-year disease-free survival (DFS), and NPLD was independent of the 5-year DFS in a further multivariate analysis; the authors finally concluded that NPLD was not an independent prognostic factor in patients with early cervical cancer [12]. However, in that study, only 5-year DFS was chosen as a prognostic indicator, and overall survival (OS) and cancer-specific survival were not included. In addition, the study did not include patients who had fewer than 11 lymph nodes removed; therefore, it cannot be concluded that NPLD and prognosis are absolutely unrelated. In 2013, Ditto *et al* investigated 526 cervical cancer patients treated with radical surgery and found that the total number of lymph nodes removed did not affect the survival of these patients, in multivariable analysis [6]. However, those authors did not divide patients into groups according to the total number of lymph nodes, which might have an impact on the result; hence, the relationship between these factors requires further study.

In their study, Shah et al. investigated data of 5522 women with stage IA2–IIA cervical cancer who underwent radical hysterectomy with lymphadenectomy; study participants were included in the Surveillance, Epidemiology, and End Results (SEER) database. In that study, the total number of lymph nodes dissected was divided into four groups: < 10, 10–20, 21–30, and > 30. The researchers found that, compared with patients who had fewer than 10 nodes removed, patients with 21–30 nodes removed were 24% less likely to die from their tumors, and those with > 30 nodes removed were 37% less likely to die. The authors concluded that more extensive lymphadenectomy was related to improved survival [13]. Similarly, Lim *et al* studied patients with FIGO stage IB–IIA cervical cancer, splitting patients into two groups according to a cutoff of 40 lymph nodes removed. The authors found that patients who had > 40 lymph nodes removed had a better prognosis, showing that the total number of lymph nodes removed had a significant effect on DFS and OS [4]. Likewise, Zhou *et al* studied 11,830 women included in the SEER database with stage IA2–IIA cervical cancer who underwent radical hysterectomy with lymphadenectomy, allocating them into four groups: 1–10, 10–20, 21–30, > 30 lymph nodes removed. The authors found that the number of lymph nodes removed

was an independent prognostic factor, which meant that the more lymph nodes were removed, the better the survival outcome [14]. From the above studies, we can conclude that the total number of lymph nodes dissected may be positively related to the prognosis of patients.

In addition, several studies have indicated that some factors (histopathological type, tumor size, neoadjuvant chemotherapy, and lymph node status) can influence the relationship between patient prognosis and the total number of lymph nodes removed.

Zhou *et al* reviewed 7920 patients with cervical squamous carcinoma and 3910 patients with cervical adenocarcinoma; all cancers were FIGO stage IA2–IIB. Those authors found that the number of lymph nodes removed was an independent positive prognostic factor in squamous carcinoma but was not related to the prognosis of patients with adenocarcinoma [14].

Lim *et al* reviewed 180 patients with FIGO stage IB–IIA cervical cancer after radical surgery, separating them into a bulky (tumor size > 4 cm) group and non-bulky (tumor size ≤ 4 cm) group. The authors found that the total number of lymph nodes removed was an independent prognostic factor with tumors > 4 cm in diameter, which meant that more extensive lymphadenectomy increased the survival of patients with bulky cervical cancer. However, with tumors < 4 cm in diameter, there was no significant correlation between the total number of lymph nodes dissected and OS or DFS [4].

When looking at surgery as the principal treatment for early cervical cancer, there are two approaches: surgery after neoadjuvant chemotherapy and direct surgical treatment. Kim *et al* divided patients into two groups using a cutoff of 20 lymph nodes removed. They found that removing a greater number of lymph nodes could improve DFS in patients without neoadjuvant chemotherapy. However, for patients who underwent radical surgery after neoadjuvant chemotherapy, there was no obvious relationship between the total number of lymph nodes removed and prognosis [15].

The study by Shah *et al* indicated that when the lymph nodes were positive, more extensive lymphadenectomy had no effect on survival; However, for women with negative lymph nodes, more extensive lymphadenectomy was associated with improved survival [13]. By contrast, Zhou *et al* found that the total number of lymph nodes removed in patients with lymph node metastasis was an independent prognostic factor for cause-specific survival and OS. In other words, the greater the number of lymph nodes excised, the better the survival outcome. However, there was no correlation between the total number of lymph nodes removed and prognosis of patients without lymph node metastasis [14]. These two studies were SEER studies; however, the study by Zhou et al. had a larger sample size (11,830 versus 5222 patients), and a larger proportion of patients were diagnosed after 2000 (76.4% versus 48%); therefore, the conclusions of Zhou *et al* may be more reliable. In the same way, Mao et al. studied 359 cases of patients with FIGO stage IA–IIB cervical cancer without lymph node metastasis, dividing them into five groups, according to the number of lymph nodes removed: < 10, 11–15,16–20, 21–25, and > 25. The authors found that when there was no lymph node metastasis, the total number of lymph nodes resected was unrelated to prognosis [16]. Similarly, Wu *et al* classified patients with early cervical cancer and without lymph node metastasis into two groups by the number of lymph nodes removed10. In univariate analysis, those authors found that the total number of lymph nodes excised was a prognostic variable in OS whereas it was irrelevant for cause-specific survival. However, multivariate analysis indicated that the total number of lymph nodes resected was unrelated to the prognosis of patients without lymph nodes metastasis [17]. It can thus be concluded that the number of lymph nodes removed is positively related to prognosis when there is lymph node metastasis, but it is not associated with prognosis when there is no lymph node metastasis. However, this conclusion requires additional and more sophisticated studies for confirmation.

Conclusions

From the current studies reviewed, we can conclude that there may be a positive correlation between the prognosis of patients with cervical cancer and the number of lymph nodes removed, in general. In the presence of lymph node metastasis, lymph nodes should be removed as extensively as possible for better prognosis, while aiming to reduce intraoperative and postoperative complications. When there is no lymph node metastasis, the prognosis of cervical cancer patients and the number of lymph nodes removed may be not related. However, whether lymph nodes are metastatic can only be completely and precisely determined by radical pelvic lymphadenectomy, to ensure their complete removal. Pelvic lymphadenectomy is the best available surgical treatment at present. As such, the procedure requires additional research, to standardize treatment. To date, there is limited research on whether neoadjuvant chemotherapy, tumor size, histological-pathological type, or lymph node status can influence the relationship between extent of lymphadenectomy and prognosis. Further multicenter, prospective studies with large samples are warranted.

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Conflict of interest

The authors declare that they have no potential conflicts of interest.

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ORIGINAL ARTICLE

Protective effects of probucol in rats with postoperative acute renal failure*

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Abstract

Objective We investigated the protective effect of probucol in rats with acute renal failure caused by various ischemia-reperfusion injuries (IRIs) after surgery.

Methods Forty male Sprague-Dawley rats were randomly divided into a sham operation group (S group), ischemia reperfusion group (IR group), probucol low-dose treatment group (probucol + IR group 1, P+ IR 1 group; probucol 250 mg/kg intragastric administration daily), and probucol high-dose treatment group (P + IR 2 group; probucol 500 mg/kg intragastric administration daily). Rats in the S and IR groups were intragastrically administered with warm water every day. After 1 week, the kidney IRI rat models were prepared, after which the rats were fed for another week, and blood, urine, and the kidney tissue specimens were retained. A series of biochemical indices, superoxide dismutase (SOD), and malondialdehyde in the serum and kidney tissues were detected, and pathological changes in renal tissue were observed.

Results Twenty-four-hour urinary protein excretion, urinary NAGase, CysC, blood urea nitrogen (BUN), and creatinine were significantly lower in the P + IR 1 and P + IR 2 groups than in the IR group ($P < 0.05$). Superoxide dismutase in the serum and renal tissue increased significantly, malondialdehyde decreased significantly ($P < 0.05$), renal pathological injury was alleviated, and the kidney index improved significantly ($P < 0.05$).

Conclusion Probucol can relieve various types of acute renal failure in postoperative rats.

Key words: probucol; ischemia-reperfusion; acute renal failure; oxidative stress

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Clinically, patients with various organ tumors often experience acute renal failure after surgery, which is among the most important causes of death after tumor surgery. The choice of effective drugs for preventing and treating acute renal failure and reducing the mortality rate of patients after tumor surgery has been widely examined. Renal ischemia-reperfusion injury (IRI) is one of the common causes of acute renal failure [1]. The kidney is an organ exhibiting high blood perfusion [2] and is very sensitive to ischemia. Excess oxygen free radicals during reperfusion further damage the renal tissue [3]. In addition to its lipid-lowering effect, probucol has anti-inflammatory, anti-oxidative, and other effects. Our previous studies confirmed that probucol has a protective effect in rats with doxorubicin nephropathy [4–5]. This study examined whether the drug also has protective effects in rats with IRI-induced acute renal failure.

Materials and methods

Experimental animals

Forty healthy male Sprague-Dawley (SD) rats weighing 180–220 g were provided by the Animal Experimental Center of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China (Experimental Animal Production License No.: SYXK (E) 2014-0046). After feeding for one week in separate cages at room temperature, urinary protein test results were all negative.

Drugs and reagents

The probucol tablets (Changtai) were produced by Chengde Jing Fu Kang Pharmaceuticals Co., Ltd. (China). The strength was 0.25 g/tablet and the lot number was 070801. Serum superoxide dismutase (SOD, lot No. 080323) and malondialdehyde (MDA, lot No. 080214) detection kits were purchased from Wuhan Kerui Biotech

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Co., Ltd. (Wuhan, China).

Grouping and model preparation

Forty rats were randomly divided into the sham operation group [S group; body weight (205 ± 8.1) g], ischemia-reperfusion group [IR group; body weight (207 ± 9.3) g], probucol low-dose treatment group [probucol + IR 1 group, P + IR 1 group; body weight (208 ± 7.9) g], and probucol high-dose treatment group [P + IR 2 group; body weight (207 ± 6.5) g]. Rats in the S and IR groups were intragastrically administered warm water daily. The P + IR 1 group was intragastrically administered probucol 250 mg/(kg•d), and the P + IR 2 group was intragastrically administered probucol 500 mg/(kg•d). IRI rat models were prepared after 1 week. The four groups of rats were fasted for 12 h before surgery. Anesthesia was performed by intraperitoneal injection of 3.5% chloral hydrate (l mL/100 g). An incision was made on the lower abdomen along the midline through the xiphoid process, the right kidney was exposed, and the right kidney vein was punctured to collect blood, after which the right kidney was excised. For the S group, only excision of the right kidney and separation of the left renal artery and vein were performed, while the left renal artery was not clipped. The left kidney was exposed, and the left renal artery and vein were separated. The left renal artery was clipped with a non-invasive arterial clip, and the color of the kidney changed from bright red to dark red. After 30 min of ischemia, the arterial clip was released and the color of the kidney changed from dark red to bright red, indicating that reperfusion was successful and the model was successfully established [6]. Normal saline replacement was administered intraperitoneally, the wound was closed layer-by-layer and covered with wet gauze, and the abdominal cavity was closed.

Specimen collection

After the models were established, the S and IR groups were intragastrically administered warm water every day, and the P + IR 1 and P + IR 2 groups were intragastrically administered probucol every day for 1 week. Rats were individually fed in metabolic cages before and on days 1, 4, and 7 after modeling. The rats were fasted without water deprivation, and 24-h urine was collected. Blood was drawn from the caudal vein. On day 7, the blood and kidney tissue samples were taken from all rats. An abdominal incision was made to remove the kidneys under sterile conditions. A portion of renal tissue was washed with normal saline at 4 °C, weighed, and ground, and the homogenate was centrifuged at 4100× g and 4 °C to obtain the supernatant for determination of SOD and MDA activity. The other part of the kidney tissue was examined under a light microscope to evaluate histomorphological changes.

Observation indicators

The kidneys were weighed, and the kidney index was calculated: (kidney weight / body weight) × 10^{-2}. Urine test: 24-h urinary protein quantification and urinary N-acetyl-beta-D-glucosaminide (NAG) enzyme were tested by the Clinical Laboratory of Wuhan General Hospital of PLA, China.

Blood test: Serum creatinine (SCr), blood urea nitrogen (BUN), and cystatin C (CysC) were measured 1 week after the models were established. Measurements were performed with an automatic biochemistry analyzer by the Clinical Laboratory of Wuhan General Hospital of PLA (China) and the average of two measurements was taken.

Serum and kidney tissue SOD and MDA detection: SOD activity and MDA content in the serum and kidney tissue were measured 1 week after the models were established. The specific procedures and calculation formulas were conducted according to the kit instructions, and the average of two measurements was taken.

Kidney morphology examination: Light microscopy: the kidney tissue was obtained, fixed with 10% formaldehyde solution, dehydrated conventionally, embedded with paraffin, and cut into 2–4-μm slices for hematoxylin and eosin (HE), Masson, and silver staining, and was observed under a regular light microscope and photographed with a professional camera.

Statistical methods

The experimental results were expressed as the mean ± the standard deviation ($\bar{\chi} \pm s$). The measurement data of multiple groups were compared by analysis of variance and categorical data were compared using χ^2 test with SPSS 11.3 software (SPSS, Inc., Chicago, IL, USA). The difference was considered statistically significant at $P < 0.05$.

Results

General conditions of rats

Compared to the S group, rats in the IR, P + IR 1, and P + IR 2 groups showed gradually slowing reactions, reduced movement, crouching and arched back, poor hair color, reduced eating and drinking, edema in the lips and limbs, and an enlarged abdomen. In the P + IR 1 and P + IR 2 groups, 1 week after treatment with probucol, food and water intake improved, body weight began to increase, reactions improved, and edema decreased, but the values were still worse than those for the S group. There was no significant improvement in the IR group.

Changes in 24-h urinary protein and urinary NAG enzyme levels before and after treatment in all groups of rats

The 24-h urinary protein and urinary NAG enzyme levels in the IR, P + IR 1, and P + IR 2 groups were significantly higher than those in the S group after modeling ($P < 0.05$). Compared to IR rats, the 24-h urinary protein and urinary NAG enzyme levels in the P + IR 1 and P + IR 2 groups were significantly reduced. The 24-h urinary protein and urine NAG enzyme levels in the P + IR 2 group were lower than those in the P + IR 1 group, but the difference was not significant ($P > 0.05$; Tables 1–2).

Changes of blood biochemistry and kidney index in each group

The levels of CysC, SCr, and BUN in the P + IR 1 and P + IR 2 groups were significantly lower than those in the IR group ($P < 0.05$). The levels of CysC, SCr, and BUN in the P + IR 2 group were lower than those in the P + IR 1 group, but the difference was not significant ($P > 0.05$). The renal index level was significantly improved in the P + IR 1 and P + IR 2 groups compared to that in the IR group (Table 3).

Contents of SOD and MDA in the serum and kidney tissue of each group

The content of MDA in serum and renal tissue of the IR group was significantly increased, while the activity of SOD was significantly decreased. Compared to the IR

Table 1 Comparison of 24-h urinary protein levels in each group ($\bar{\chi} \pm s$, mg)

Group (Number of animals)	Before modeling	1 day after modeling	4 days after modeling	7 days after modeling
S (10)	6.32 ± 1.27	6.45 ± 1.08	6.62 ± 1.35	6.62 ± 1.35
IR (10)	6.71 ± 1.85	182.12 ± 15.24^{ab}	279.12 ± 19.61^{ab}	373.12 ± 22.61^{ab}
P + IR 1 (10)	6.64 ± 1.57	132.36 ± 19.27^{abc}	210.17 ± 13.26^{abc}	302.38 ± 21.74^{abc}
P + IR 2 (10)	6.62 ± 1.43	125.65 ± 20.36^{abcd}	203.45 ± 14.05^{abcd}	294.45 ± 21.05^{abcd}

Note: Compared to the value before modeling, $^{a}P < 0.05$; compared to the S group, $^{b}P < 0.05$; compared to the IR group, $^{c}P < 0.05$; compared to the P + IR 1 group, $^{d}P > 0.05$

Table 2 Comparison of urine NAG enzyme levels in each group ($\bar{\chi} \pm s$, U/L)

Group (Number of animals)	Before modeling	1 day after modeling	4 days after modeling	7 days after modeling
S (10)	20.4 ± 2.3	20.7 ± 1.9	21 ± 2.4	22 ± 2.2
IR (10)	20.5 ± 2.1	49.1 ± 3.9^{ab}	74.4 ± 4.3^{ab}	90.4 ± 5.7^{ab}
P + IR 1 (10)	20.3 ± 2.4	40.5 ± 3.4^{abc}	53.7 ± 4.1^{abc}	76.1 ± 5.2^{abc}
P + IR 2 (10)	20.3 ± 2.2	38.6 ± 3.1^{abcd}	49.1 ± 3.8^{abcd}	72.5 ± 4.9^{abcd}

Note: Compared to the value before modeling, $^{a}P < 0.05$; compared to the S group, $^{b}P < 0.05$; compared to the IR group, $^{c}P < 0.05$; compared to the P + IR 1 group, $^{d}P > 0.05$

Table 3 Effects on blood biochemistry and kidney index in each group ($\bar{\chi} \pm s$)

Group (Number of animals)	CysC (mg/L)	BUN (mmol/L)	SCr (mmol/L)	Kidney index
S (10)	0.87 ± 0.26	7.23 ± 1.13	57.38 ± 11.72	0.82 ± 0.35
IR (10)	4.68 ± 1.75^{a}	10.58 ± 2.35^{a}	146.13 ± 10.45^{a}	1.26 ± 0.35^{a}
P + IR 1 (10)	3.01 ± 0.84^{ab}	9.5 ± 1.22^{ab}	102.35 ± 9.82^{ab}	1.054 ± 0.43
P + IR 2 (10)	2.91 ± 0.73^{abc}	9.4 ± 1.02^{abc}	98.16 ± 9.74^{abc}	1.04 ± 0.41^{abc}

Note: Compared to the S group, $^{a}P < 0.05$; compared to the IR group, $^{b}P < 0.05$; compared to the P + IR 1 group, $^{c}P > 0.05$

Table 4 SOD and MDA contents in the serum and kidney tissue of each group ($\bar{\chi} \pm s$)

Group (Number of animals)	Serum SOD (U/mL)	Kidney tissue SOD (U/mL)	Serum MDA (nmol/mL)	Kidney tissue MDA (nmol/mL)
S (10)	60.53 ± 11.63	97.85 ± 16.49	2.25 ± 0.73	2.87 ± 1.25
IR (10)	32.76 ± 7.72^{a}	49.12 ± 11.46^{a}	4.61 ± 1.26^{a}	4.91 ± 1.67^{a}
P + IR 1 (10)	52.13 ± 10.79^{ab}	84.28 ± 16.47^{ab}	3.96 ± 1.82^{ab}	4.11 ± 1.52^{ab}
P + IR 2 (10)	49.02 ± 10.24^{abc}	82.31 ± 15.98^{abc}	3.87 ± 1.73^{abc}	3.97 ± 1.35^{abc}

Note: Compared to the S group, $^{a}P < 0.05$; compared to the IR group, $^{b}P < 0.05$; compared to the P + IR 1 group, $^{c}P > 0.05$

group, the content of MDA in the serum and kidney tissue of the P + IR 1 and P + IR 2 groups decreased significantly, and SOD increased significantly ($P < 0.05$). The content of MDA in the P + IR 2 group was lower than that in the P + IR 1 group and the SOD was increased, but the difference was not significant ($P > 0.05$; Table 4).

Changes of kidney pathomorphology in each group

Under light microscopy, the glomeruli, renal tubules, and interstitium were normal in the S group. The IR group was mainly characterized by renal tubular injury. HE staining showed slight hyperplasia of the glomerular mesangial matrix, widened renal interstitium infiltrated by a considerable volume of inflammatory cells, some atrophied renal tubules, and shedding tubular epithelial cells; a considerable volume of red blood cells and white blood cells in the renal interstitium, as well as blood stasis in the small interstitial blood vessels were observed. Masson + silver staining showed a focal distribution of interstitial fibrosis. The pathological changes of rats in the P + IR 1 and P + IR 2 groups were significantly lower

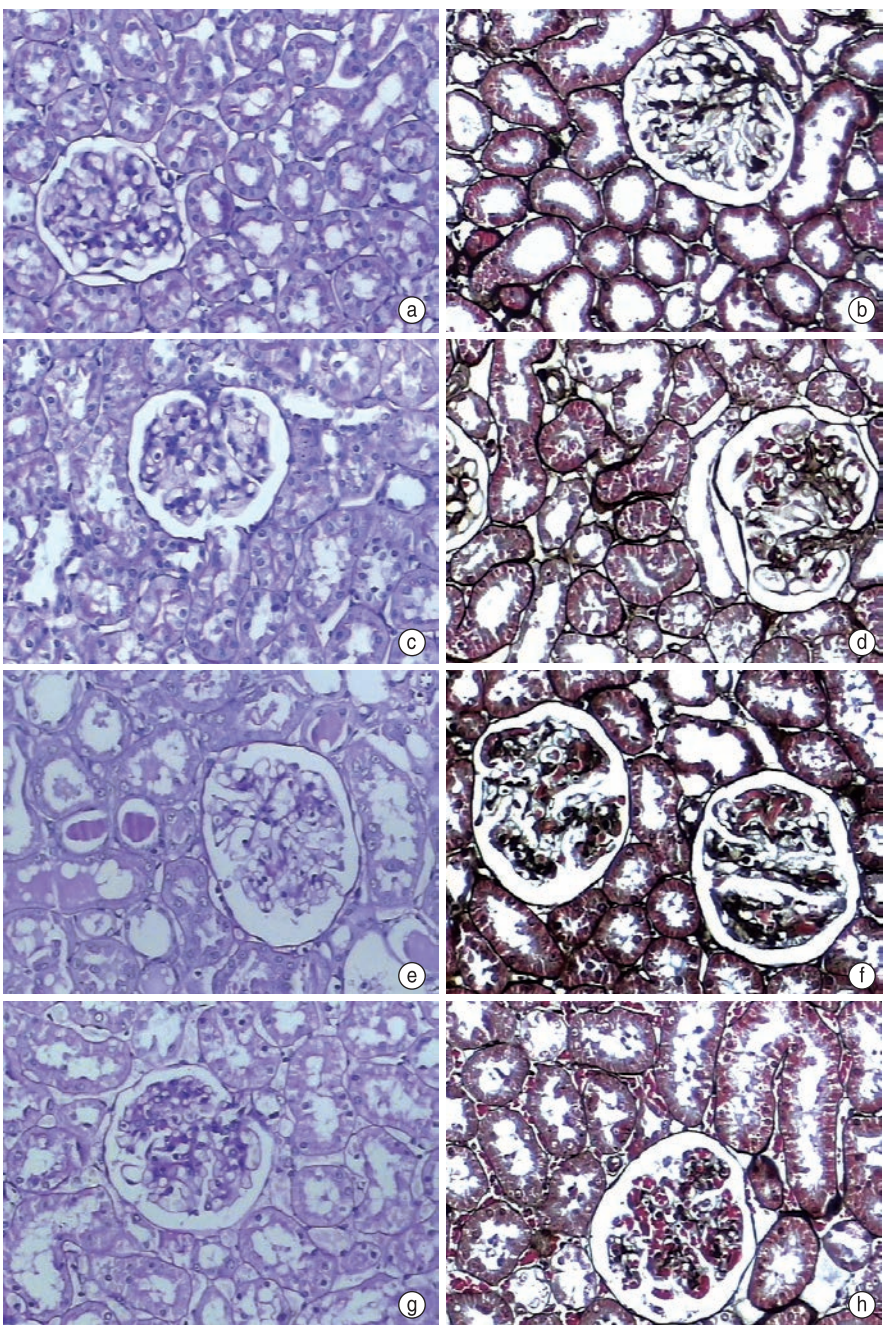

Fig. 1 Pathomorphological changes in the kidneys of rats in each group (×400). (a) HE staining of the S group; (b) Masson + silver staining of the S group; (c) HE staining of the IR group; (d) Masson + silver staining of the IR group; (e) HE staining of the P + IR 1 group; (f) Masson + silver staining of the P + IR 1 group; (g) HE staining of the P + IR 2 group; (h) Masson + silver staining of the P + IR 2 group

than those in the IR group. The glomerular structure was relatively normal, and renal interstitial lesions were mild (Fig. 1).

Discussion

As patients with advanced malignant tumors, particularly elderly patients, have reduced immune function and degenerative changes in the function of various organs, poor tolerance to surgery and acute renal failure after surgery are common. This is mainly because blood loss after surgery leads to effective circulating blood volume, microcirculation, renal ischemia, and hypoxia injury; vital organs of the body, including the kidneys, have reduced compensatory ability; and surgical wounds lead to increased oxidative stress and increased inflammatory reactivity. Studies have shown that for acute renal failure in elderly patients with malignant tumors, the risk of death is significantly increased. Therefore, effective measures must be taken to prevent and treat acute renal failure in patients after tumor surgery.

The kidney is a high-blood-perfusion organ, whose blood flow accounts for 20%–25% of the blood flow in the body and is very sensitive to ischemia. Kidney IRI is one of the common causes of acute renal failure. The pathogenesis of renal IRI is complex and is currently thought to be mainly related to the following factors: renal cell apoptosis, oxidative stress response, inflammatory response, endothelial dysfunction, and impaired cellular energy metabolism [7–8]. Increased oxidative stress in the kidney is particularly critical. After IRI in the kidney, more oxygen radicals are produced through the xanthine oxidase pathway. Activated oxygen radicals act on the unsaturated fatty acids in the cell membrane of the kidney, resulting in lipid peroxidation and a large amount of MDA. MDA is a commonly used indicator that reflects the body's lipid peroxide content and degree of attack by superoxide radicals [9]. SOD is a major antioxidant enzyme in the body and kidney tissues. It removes superoxide anions from the body, reduces oxidative stress damage in cells, and repairs damaged cells [10–11].

Probucol was used clinically as a lipid-lowering drug in the 1970s. In recent years, studies have shown that probucol also has good anti-oxidative stress, anti-inflammatory, and endothelial function improvement effects. In this study, 24-h urinary protein, urinary NAGase, CysC, SCr, BUN, and other indicators were significantly elevated in rats after IRI, renal pathological lesions were evident, renal index deteriorated, and acute renal failure occurred. After treatment with low-dose and high-dose, the levels of MDA in the serum and renal tissue of the P + IR 1 and P + IR 2 groups were significantly decreased, and SOD activity was significantly increased ($P < 0.05$). The drug improved oxidative stress in the kidneys and restored the balance between oxidation and anti-oxidation. In terms of renal function, 24-h urinary protein quantification, urine NAGase, CysC, SCr, BUN, and other indicators were significantly decreased ($P < 0.05$), indicating that renal failure was improved. In addition, renal pathological injury and the kidney index also significantly improved. These results demonstrate that probucol reduces acute renal failure. Our previous studies confirmed that heme oxygenase-1 (HO-1) has a protective effect on the kidneys in rats with chronic renal insufficiency [12], while probucol induces HO-1 expression and increases the activity of HO-1 [13], which may be another important mechanism in renal protection.

In summary, when acute renal failure occurs after surgery for malignant tumors, probucol has good alleviation and treatment effects. The mechanism may involve lipid regulation, anti-oxidative stress, anti-inflammation, endothelial function improvement, etc. Probucol shows potential for clinical application.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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ORIGINAL ARTICLE

Correlation between sodium-iodide symporter expression and circulating tumor cell positivity in differentiated thyroid carcinoma*

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Abstract

Objective We investigated the correlation between the expression of the sodium-iodide symporter (NIS) and the detection of circulating tumor cells (CTCs) in differentiated thyroid carcinoma (DTC).

Methods NIS expression in differentiated thyroid and the positive rate of CTCs in the peripheral blood were determined by immunohistochemistry S-P and flow cytometry from the records of 172 cases of differentiated thyroid carcinoma.

Results Seventy-six cases (44.2%) expressed NIS in the differentiated thyroid and 63 cases (36.6%) were positive for CTCs in the peripheral blood. There was a significant difference between N0 and N1 in the expression of NIS (χ^2 = 6.015, P = 0.014) and the positive rate of CTCs (χ^2 = 14.035, P = 0.001). N0 and N1 also differed significantly in the expression of NIS (r = -0.383, -0.610, P = 0.002, < 0.001). The differences in the NIS expression, but not in the positive rate of CTCs, were significant among the different pathological subtypes (χ^2 = 7.897, P = 0.005; χ^2 = 1.455, P = 0.228, respectively). There was a significant negative correlation between the highly differentiated type and intermediate differentiation type both in the expression of NIS and positive rate of CTCs (r = -0.591, -0.443, P < 0.001, P = 0.002).

Conclusion There was a significant negative correlation between the expression of tissue NIS and positive rate of CTCs in the peripheral blood in DTC. The malignancy level and lymph node metastasis in differentiated thyroid carcinoma were negatively correlated with NIS expression and positively correlated with the positive rate of CTC.

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Keyword: differentiated thyroid cancer (DTC); sodium-iodide symporter (NIS); circulating tumor cell flow cytometry

In recent years, the sodium-iodide symporter (NIS) and circulating tumor cells (CTCs) have been studied in thyroid carcinoma. These factors have gradually become a reference index for cancer diagnosis, therapy evaluation, and prognosis [1-3]. However, whether CTCs can be used to indicate individualized treatment of tumors has not been widely examined. In this study, we evaluated the correlation between NIS expression and the positive rate of CTCs in differentiated thyroid carcinoma. We have also discussed herein the radioiodide treatment of CTCs in differentiated thyroid carcinoma.

Materials and methods

Subjects

From February 2008 to October 2013, 172 cases of differentiated thyroid carcinoma were enrolled in Gansu Provincial Tumor Hospital, China. There were 38 males and 134 females, age 14–73 years, with a median age of 36.7 years. All patients were pathologically diagnosed according to uniform standards for diagnosis and treatment [4–5]. There were separated into 4 groups based on tumor size: 28 cases of T1, 67 cases of T2, 46 cases of T3, and 31 cases of T4. Sixty-four cases were lymph node stage N0 and 108

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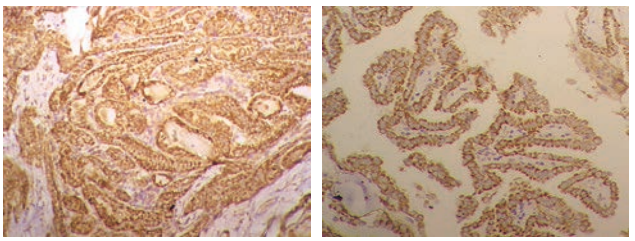

Fig. 1 Immunohistochemical detection of NIS-positive expression in thyroid cancer (× 400)

cases were lymph node stage N1. Well-differentiated tumors were diagnosed in 124 cases and intermediated tumors were diagnosed in 48 cases. Before obtaining blood samples, all patients provided written informed consent. Patients did not receive chemotherapy, radiation therapy, or radionuclide therapy before blood sample collection.

Equipment and reagents

The FACS Calibur flow cytometer was from BD Biosciences (USA). Anti-CK19/FITC were purchased from eBioscience (USA). The Muc1/CD227 antibody was from Abcam (UK). Breaking agent (No. 641776) and FACS hemolysin (No. 349202) were purchased from BD Biosciences. NIS mouse anti-human monoclonal antibody (MAB3562) was from Millipore (USA). The S-P kit was from Boster Bioengineering Co., Ltd. (China).

Immunohistochemical analysis

All slides were stained with immunohistochemistry S-P by immunohistochemistry (IHC). Analysis was conducted by one pathologist (AB) who was blinded to the related clinical information. NIS expression was used as positive control and primary antibody substituted for phosphate-buffered saline was used as a negative control. NIS-positive expression was mainly observed on the cell membrane, and positive expression was claybank or brown, but the negative result showed no color. IHC results were analyzed by two pathologists, defined in a semiquantitative manner, using the I × E product method[6]. Each slice was randomly selected from five visual fields under a microscope (× 400), and each field had an average of 200 cells. The I-Grading scale was as follows: 0, same as the background or weak staining; 1+, pale yellow staining; 2+, yellow or claybank staining; 3+, brown staining. The E-Grading scale was as follows: 0, ≤ 10%; 1+, 10–25%; 2+, 26–50%; 3+, ≥ 51%. I × E integral evaluation: ≤ 1, negative (-); 1–4, weakly positive (+); ≥6, strongly positive (+ +) (Fig. 1).

Detection of CTC in peripheral blood

Five milliliters of venous blood were collected 1 week after surgery, 10% EDTA-Na_2 was used for anticoagulation, and marked respectively by anti-CK19 and MUC1/CD227. A FACS Calibur flow cytometer was used, setting forward scatter and side scatter to eliminate the various fragments and granulum from the sample. The results excluded single positive cases of cytokeratin 19 (CK19) or polymorphic epithelial mucin1 (MUC1). Both CK19 and MUC1 were expressed as CTC-positive cases in the peripheral blood.

Statistical analysis

Statistical analysis was performed using SPSS 22.0 software (USA), and data from two groups were compared using Fisher's exact test or χ^2 analysis. Statistical analysis was performed by using the Pearson method, to test the significance, the risk level was set to 0.05.

Results

NIS and CTC-positive expression (Table 1)

In 172 cases of differentiated thyroid carcinoma patients, 76 cases were NIS-positive in thyroid cancer tissue (44.2%), while 63 cases were CTC-positive in the peripheral blood (36.6%).

Differences in gender, age (≤ 45 years old, > 45 years old), NIS-positive rates, and CTC-positive rates in thyroid cancer patients were not significant.

The NIS-positive rates were significantly different between lymph node stage N0 and N1 (χ^2 = 6.015, P = 0.014), and the CTC-positive rates were significantly different (χ^2 = 14.035, P = 0.001). The NIS-positive rates were significantly different among the pathological subtypes, while CTC-positive rates were significantly different (χ^2 = 1.455, P = 0.228).

Correlation between NIS-positivity and CTC-positivity

There was a significant negative correlation in lymph node stage N0 between the NIS-positive rates and CTC-positive rates (r = -0.383, P = 0.002), and in lymph node stage N1 (r = -0.610, P < 0.001). There was also a significant negative correlation in highly differentiated between cases the NIS-positive rates and CTC-positive rates (r = -0.591, P < 0.001), and in intermediate cases (r = -0.443, P = 0.002) (Table 2).

Discussion

NIS is a class of membrane proteins on the basement membrane of thyroid follicular epithelial cells, which mediates active transport of iodine in the thyroid. Their main role is to promote the reverse concentration

Table 1 NIS and CTC expression in differentiated thyroid carcinoma

	n	NIS expression		χ^2	*P*	CTC expression		χ^2	*P*
		(+)	(-)			(+)	(-)		
Sex									
Males	38	15 (39.5%)	23 (60.5%)	0.49	0.508	15 (39.5%)	23 (60.5%)	0.170	0.680
Females	134	61 (45.5%)	73 (54.5%)			48 (35.8%)	86 (64.2%)		
Age (years)									
≤ 45	98	45 (45.9%)	53 (54.1%)	0.277	0.599	34 (34.7%)	64 (65.3%)	0.367	0.545
> 45	74	31 (41.9%)	53 (54.1%)			29 (39.2%)	45 (60.8%)		
Lymph node stage									
N0	64	36 (56.3%)	28 (43.7%)	6.015	0.014	12 (18.75%)	52 (81.25%)	14.035	< 0.001
N1	108	40 (37.0%)	68 (63.0%)			51 (47.22%)	57 (52.78%)		
Pathologically									
Highly differentiated	124	63 (50.8%)	61 (49.2%)	7.897	0.005	42 (33.87%)	82 (66.13%)	1.455	0.228
Intermediate	48	13 (27.1%)	35 (72.9%)			21 (43.75%)	27 (56.25%)		

Table 2 Comprehensive analysis of NIS-expression and CTC-positive results in DTC

	n	Conditions				*P*	*r*
		CTC(-) NIS(+)	CTC(-) NIS(-)	CTC(+) NIS(-)	CTC(+) NIS(+)		
Lymph node stage							
N0	64	34	18	10	2	0.002	-0.383
N1	108	37	20	48	3	< 0.001	-0.610
Pathologically							
Highly-differentiated	124	59	23	38	4	< 0.001	-0.591
Intermediate	48	12	15	20	1	0.002	-0.443

gradient of thyroid to transport inorganic iodine and participate in the biosynthesis of thyroid hormone. Iodide uptake in thyroid follicular cells mainly depends on NIS function and structural integrity. DTC has some functions of normal thyroid cells and maintains their iodide uptake ability. NIS expression is the basis of diagnosis and radioiodide therapy for DTC. Diagnosis and treatment can be determined by detecting the ability of NIS to take up iodide for DTC. However, approximately 30% of patients exhibit "dedifferentiation" in tumor cells because of tumor recurrence, metastasis, chemotherapy, radiotherapy, and ^{131}I therapy. Dedifferentiated thyroid cancer shows a loss of NIS-expression, which is the loss of function of the "iodine pump", resulting in the failure of ^{131}I therapy [7–8]. CTCs are tumor cells that enter the blood circulation through a primary lesion or metastasis, and may enter the circulating blood before forming a solid tumor lesion [9]. Circulation blood is the only method of causing distant metastasis of tumor cells; CTC and hematogenous metastasis of tumors are directly related, and thus the detection of CTCs facilitates the early diagnosis of tumor metastasis, monitoring of postoperative recurrence and metastasis of the tumor, and the choice of individualized treatment strategies [10–11]. According to the results of large sample multivariate analysis, CTCs can be used as independent prognostic factors for tumor treatment as well as dynamic monitoring of CTC changes to predict tumor curative effects and tumor progress earlier in patients with tumors in the peripheral blood of CTC. Surgery alone cannot achieve effect a radical cure, and thus systemic adjuvant therapy is required. Therefore, in-depth studies of CTC can improve the understanding of the mechanisms of tumor metastasis and provide a new basis for early treatment of anti-tumor metastasis [12–13] .

This study showed that patients with thyroid cancer lymph node metastasis N0 and N1, tumor tissue NIS showed significantly positive expression and peripheral blood showed significantly CTC-positive differences (P = 0.041, < 0.001). NIS expression in patients in the N0 group was significantly negatively correlated with the CTC-positive rate (r = 0.383, P = 0.383), while NIS expression in the N1 group showed a significantly negative correlation with the CTC-positive rate (r = 0.610, P < 0.001). These results tentatively suggest that thyroid cancer cells with a loss of NIS expression have actively growing tumors; as the size and internal pressure of the tumor increases, lymph node metastasis occurs, and tumor cells may be removed from the tumor and enter the peripheral circulation. From another perspective, after malignant tumors show metastatic lymph node metastasis, tumor cell proliferation and metabolism are accelerated in the lymphoid tissue. Tumor cells not only metastasize to another lymph node through the lymph, but also enter the blood circulation, resulting in CTC multiplication in the peripheral blood [14]. These thyroid tumor tissues in which NIS showed loss of expression lost the iodine function of NIS, and 131I showed poor efficacy and poor prognosis. The NIS expression-positive rate between various pathological subtype differences were significant (P = 0.005), but there was no significant difference in the rate of positive of

CTCs (P = 0.228). NIS expression in highly differentiated and intermediate tumors was significantly negatively correlated with the CTC-positive rate (r = 0.591, 0.443, P < 0.001, P = 0.002). In the pathological subtype of differentiated thyroid carcinoma, the development of high differentiation was slow, malignant grade, and showed a good prognosis, with a 10-year survival rate of over 85% [15]. Highly differentiated thyroid cancer cells expressed NIS, and radionuclide irradiation therapy was positive and the prognosis was good for NIS-positive expression, but the CTC-positive rate was low in these patients. The deteriorated and lymph node metastasis of thyroid cancer was positively correlated with the peripheral blood CTC-positive rate and negatively correlated with tumor tissue NIS expression. When peripheral blood CTC was positive, tumor tissues lost NIS expression.

This study showed that detection of CTCs in the peripheral blood can be used to preliminarily evaluate NIS expression and predict efficacy, which is very important for radioiodide therapy for DTC. CTCs are promising new circulating markers for DTC, which have excellent practical value of efficacy assessment and prognosis.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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CASE REPORT

Recurrent ascites due to spontaneous intraperitoneal bladder rupture after pelvic radiation therapy for cervical cancer

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Abstract

Radiation cystitis is one of the major complications following radiotherapy for cervical cancer. However, spontaneous intraperitoneal bladder rupture as a result of radiation cystitis following radiotherapy for cervical cancer is extremely rare. Case presentation: We report a 52-year-old patient who received radiation therapy for cervical cancer 15 years prior to presentation. Eight years prior to presentation, she developed recurrent abdominal distension, oliguria, and ascites. Following ascites drainage and supportive treatment, all symptoms were relieved. However, all symptoms subsequently recurred every few months. The patient underwent exploratory laparotomy twice. The first exploratory laparotomy in July 2015 found no specific abnormalities. The second exploratory laparotomy in November 2016 found an intraperitoneal bladder rupture, and the patient underwent surgical repair. The ascites subsequently resolved. Conclusion: The occurrence of spontaneous intraperitoneal bladder rupture after radiation therapy for cervical cancer is rare. The prognosis is good when diagnosis and treatment are prompt.

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Key words: radiation cystitis; spontaneous intraperitoneal bladder rupture; recurrent ascites; cervical cancer

Urinary bladder rupture is mostly associated with trauma, chronic bladder disease, or bladder outflow obstruction. Nontraumatic, spontaneous intraperitoneal bladder rupture, which is associated with pelvic radiation therapy, is rare in cervical cancer patients. The diagnosis of urinary bladder rupture may be difficult due to unreliable history and variable presentation. Spontaneous intraperitoneal bladder rupture is commonly initially misdiagnosed, and sometimes can be a life-threatening event.

Case presentation

A 52-year-old woman presented to the hospital (Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China) with abdominal distension, oliguria, and ascites for the first time in January 2010. She denied any history of trauma. She was diagnosed with cervical cancer in 2003. She underwent radical hysterectomy and pelvic lymph node dissection on March 24, 2003, and was diagnosed with squamous carcinoma of the cervix (stage IIb). She received postoperative pelvic concurrent chemo-radiotherapy, including pelvic radiation (Dt 4600 cGy/23 F) and brachytherapy (Dt 1700 cGy/3 F), then regular follow-up. After a 5-year follow-up period, there was no significant recurrence, the patient was asymptomatic, and regular follow-up was discontinued.

In January 2010, the patient was admitted to the hospital (Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China) because of abdominal distension and pain, decreased urine output, and a large amount of ascites. On examination, her temperature, blood pressure,

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and heart rate were normal. Chest auscultation showed a regular heart beat without murmur and clear breath sounds without crackles. The abdomen was grossly distended with free fluid, and she had hypoactive bowel sounds. There were no features suggestive of peritonitis, no palpable liver or spleen, and no percussion pain in the kidney area. No abnormalities were found on gynecologic examination. The diagnosis at admission was suspected ascites of unknown cause and cervical cancer. Blood urea nitrogen and serum creatinine were slightly increased. The complete blood cell count, liver function, and coagulation tests were normal, and tumor marker levels, including carcinoembryonic antigen (CEA), squamous cell carcinoma antigen (SCCA), CA 153, CA 125, and CA 19-9 were also normal. Ultrasound examination showed a rough bladder wall and a large amount of abdominal and pelvic effusion. Computed tomography (CT) showed a large amount of ascites without evidence of malignancy or any obstruction. No obvious abnormalities were found on cystoscopy, other than pale bladder mucosa. Positron emission tomography PET/CT showed abnormally elevated fluorodeoxyglucose uptake in both the abdominal and pelvic cavities, with strong suspicion of metastatic lesions. Routine biochemical tests and pathological examination of the ascites fluid revealed the presence of red blood cells, lymphocytes, and mesothelial cells, but no cancer cells. The patient was diagnosed with incomplete malignant bowel obstruction, and was discharged after improvement with symptomatic and supportive treatment. From 2010 to 2015, the patient had recurrent abdominal pain and distension, with different amounts of ascites. No obvious abnormalities were found on CT and cystoscopy. The symptoms were relieved with ascites drainage and supportive treatment.

In July 2015, the patient was readmitted with the same symptoms, including abdominal pain, lower abdominal distension, and oliguria. PET/CT examination showed no specific abnormalities. A multidisciplinary team (MDT) consisting of an oncologist, gynecologist, gastroenterologist, and radiologist, decided to perform laparoscopic exploration, but no obvious abnormality was found. In 2015 and 2016, the patient experienced intermittent abdominal pain and a large amount of ascites. Symptoms usually occurred without apparent cause. The nonspecific treatment included ascites drainage and symptomatic and supportive treatment, since the cause of the ascites had not been identified. In November 2016, the patient presented with the same symptoms, and underwent exploratory laparotomy for the second time. The bladder wall was found to be extremely thin, and a very tiny fissure was found on the wall. After repair of the ruptured bladder, the patient recovered, and has not experienced ascites since then.

Discussion

Our case highlights three interconnected phenomena: radiation cystitis, recurrent ascites, and spontaneous bladder rupture.

Radiation cystitis is a common complication of cervical cancer after pelvic radiotherapy [1]. According to the time of occurrence and severity, radiation cystitis may be divided into three types: acute radiation cystitis, chronic radiation cystitis, and radiation-induced bladder fistula [2–3]. Acute radiation cystitis occurs during or soon after radiation treatment, usually within 6 months. It is mostly characterized by increased urinary frequency and urgency, with gross or microscopic hematuria [4], and is usually self-limited and generally managed conservatively [5]. Chronic radiation cystitis accounts for 80% of cases of radiation cystitis [6], and can develop 6 months to 20 years after radiation therapy. The main presenting symptom is hematuria, which may vary from mild to severe, life-threatening hemorrhage [5, 7]. Radiation-induced bladder fistula is often associated with the radiation dose, and can occur in some severe cases.

Spontaneous intraperitoneal bladder rupture is rare in cervical cancer patients who undergo radiation therapy, but can be life-threatening [8]. The most common causes of bladder rupture are blunt trauma, chronic bladder disease, or bladder outflow obstruction; other possible reasons include surgical procedures and irradiation to the pelvis [9–12]. Accurate diagnosis of spontaneous intraperitoneal bladder rupture is difficult before surgery and is often delayed in the absence of history of trauma or preexisting chronic bladder disease. Symptoms and signs of spontaneous intraperitoneal bladder rupture can be nonspecific and misleading [13–15]. Patients usually present with an acute abdomen, abdominal distension, oliguria/dysuria, and hematuria [12–13]. Symptoms may be insidious in onset, presenting only as ascites or acute renal failure[14, 16–18]. Ultrasonography and CT may miss most intraperitoneal bladder ruptures. CT cystography may help to diagnose bladder rupture, but it is difficult to identify tiny fissures [19]. The gold standard for the diagnosis of intraperitoneal bladder rupture is exploratory laparotomy. However, this operation is invasive for a patient without serious complications. Measuring urea and creatinine levels in ascites and serum is a simple and noninvasive diagnostic test, and an ascites-to-serum creatinine ratio > 1.0 usually supports the diagnosis of spontaneous intraperitoneal bladder rupture [20–21]. The conservative treatment of spontaneous intraperitoneal bladder rupture consists of antibiotics and percutaneous peritoneal drainage for patients with a history of pelvic irradiation [22–23]. For recurrent cases, or patients with severe symptoms after ineffective conservative therapy, immediate surgery, with repair of the urinary bladder in

2 layers, is strongly recommended [24–25]. After repair of the bladder, prolonged drainage is required, and patients must be educated to avoid bladder overdistension, because of increased risk of reperforation [26].

As described by Addar *et al* in 1996 [27], treatment of spontaneous bladder rupture must be individualized but should be based upon 6 principles as follows: (1) the defect must be identified and confirmed; (2) the peritoneal cavity should be thoroughly lavaged; (3) the defect should be widely excised; (4) reconstitution of the intact bladder should be performed using tissue with an intact blood supply, especially in radiated areas; (5) adequate healing with prolonged bladder drainage and prophylactic antibiotics should be promoted; and (6) primary or recurrent malignant disease should be excluded.

Seven years after surgery and radiotherapy, our patient began to experience intermittent abdominal pain and a large amount of ascites. During the next 6 years, although she had undergone cystoscopy, PET/CT, and even laparotomy, there were no positive findings to explain all the symptoms. We did not perform a peritoneal fluid analysis of urea and creatinine levels because we did not initially consider the possibility of spontaneous intraperitoneal bladder rupture. It was not until the second exploratory laparotomy that the bladder wall was found to be significantly thin, with a tiny localized fissure. After the bladder was repaired in 2 layers, the patient recovered and has not experienced abdominal pain and ascites again.

This case is rare. The patient was admitted to the hospital repeatedly over 6 years with recurrent ascites, but no peritonitis or acute renal failure. This patient was a dancer, and often delayed bladder emptying due to occupational reasons. Overdistension is hazardous for a bladder that has undergone radiation therapy and will aggravate the occurrence of all complications. Since the rupture site was extremely small and pelvic adhesions developed after surgery and radiotherapy, the fissure was not detected on cystoscopy and initial laparoscopic exploration.

This case shows that when a cervical cancer patient experiences ascites after radiotherapy, we should think of the possibility of rupture of the bladder [28]. For patients with recurrent ascites, cytology should be performed and urea and creatinine levels in the ascites fluid should be tested. Spontaneous intraperitoneal bladder rupture should always be considered in the differential diagnosis of patients who present with abdominal distension, oliguria, ascites, and increased levels of urea and creatinine in serum and/or peritoneal fluid aspirate [29]. The prognosis is good when diagnosis and treatment are prompt.

Conclusion

Spontaneous intraperitoneal bladder rupture is a rare cause of ascites, but when a patient presents with ascites and oliguria of unknown cause, especially with a history of radiation therapy to the pelvis, the possibility of spontaneous intraperitoneal bladder rupture should be considered in the differential diagnosis. To avoid the risk of death, prompt and precise diagnosis are mandatory. After proper surgical treatment, patients must be educated regarding bladder emptying, to prevent overdistension. We hope our case report heightens awareness of spontaneous intraperitoneal bladder rupture, so that these patients can be diagnosed promptly and treated appropriately to obtain the best possible outcomes.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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CASE REPORT

Small cell carcinoma of the gastric remnant: a case report

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Abstract

Objective Small cell carcinoma (SCC) is mostly found in the lungs. It is extremely rare in the gastric remnant. Here, we report a case and review the literature in order to improve the diagnosis and treatment of SCC of the gastric remnant.

Methods We report a case of SCC of the gastric remnant in a 71-year-old male Chinese patient who presented with epigastric pain, acid regurgitation, and belching and who underwent Billroth II gastrectomy more than 38 years ago.

Results Physical examination showed no obvious abnormalities. Laboratory data were within normal limits, except for anemia. Pathology of the mass showed a protruded tumor measuring 5.0 × 5.0 × 2.5 cm at the anastomotic edge of the gastric remnant that infiltrated through the full wall of the stomach; this was confirmed by immunohistochemical staining for cytokeratin [CK (-)], leukocyte common antigen (LCA) (+), synaptophysin (+), CD56 (+), and Ki-67 (+ > 50%).

Conclusion SCC of the gastric remnant is extremely rare, although the pathology, symptoms, diagnosis, treatment, and prognosis of SCC are similar to those of gastric SCC. Although the standard treatment of SCC of the gastric remnant remains unclear, effective surgical resection and subsequent multiagent chemotherapy should be performed for long-term survival. Our case shows the efficacy of tegafur-gimeracil-oteracil-potassium capsule chemotherapy. Examination of a large series is required to determine the optimal treatment strategy for SCC of the gastric remnant.

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Keywords: small cell carcinoma (SCC); gastric; stump cancer; gastric remnant; gastric carcinoma

Gastric small cell carcinoma (GSCC), a malignant cancer characterized by invasion and metastasis [1], is extremely rare in the gastric remnant. SCC of the gastric remnant is similar to GSCC in terms of clinicopathologic features and biological characteristics. Histological and immunohistochemical (IHC) analyses are helpful for pathological diagnosis [2]. SCC of the gastric remnant is extremely difficult to diagnose and is associated with a poor prognosis, and the standard treatment remains unknown due to its rarity [3].

Here, we report a patient with SCC of the gastric remnant who remains alive more than three years after treatment that included a combination of surgery and chemotherapy in order to improve the diagnosis and treatment of SCC of the gastric remnant.

Case report

The patient, a 71-year-old man, was referred to our hospital with epigastric pain, acid regurgitation, and belching for three months, without nausea, emesis, fever, or chills. Billroth II gastrectomy was performed 38 years ago. Physical examination showed a body temperature of 36.5°C, pulse rate of 74 beats/min, and blood pressure of 100/70 mmHg. A scar of about 12 cm was observed in the middle of his abdomen; his abdomen was soft and nontender, and the liver and spleen were not palpable. Laboratory data were within normal limits, except for anemia that was indicated by a hemoglobin level of 108 g/L and a hematocrit level of 22.6%. The levels of tumor markers, including carcinoembryonic antigen (CEA), alpha fetoprotein, and carbohydrate antigen 19-9 were

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also within normal limits (2.77 ng/mL, 1.75 μg/L, and 23.77 U/mL, respectively).

Gastroscopy revealed a post-gastrectomy appearance and an ulcerative lesion measuring approximately 2.0×2.5 cm on the anastomotic edge of the gastric remnant (Fig. 1). The pathological diagnosis of the biopsy specimen indicated a poorly differentiated SCC. Laparoscopy-assisted total gastrectomy was performed successfully on November 21, 2014, and the patient recovered well. Pathological examination of the mass showed a protruded tumor measuring 5.0 × 5.0 × 2.5 cm at the anastomotic edge of the gastric remnant, which infiltrated the full wall of the stomach but had not invaded the incised edge and omentum majus. In addition, none of the perigastric lymph nodes showed metastasis. Microscopically, the mass showed diffuse proliferation of the small cells with scanty cytoplasm and hyperchromatic nuclei (Fig. 2). IHC analysis showed CK (-), leukocyte common antigen (LCA) (+), synaptophysin (Syn) (+), CD56 (+), and Ki-67 (+ > 50%) (Fig. 3). Histomorphology and immunohistochemistry of this patient were consistent with those for SCC. The patient underwent adjuvant chemotherapy that included four courses of three tegafur, gimeracil, oteracil, and potassium capsules twice daily for two weeks with a one-week break. Up to now, the patient has been free of recurrence, and long-term, regular follow-up is in progress.

Discussion

SCC is a malignant cancer frequently observed in the lungs, whereas extrapulmonary small cell cancer (EPSCC) is uncommon. EPSCC has been reported in the

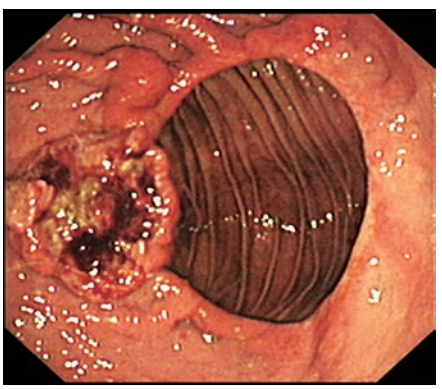

Fig. 1 Gastroscopy showed an ulcerative lesion measuring approximately 2.0 × 2.5 cm on the anastomotic edge of the gastric remnant.

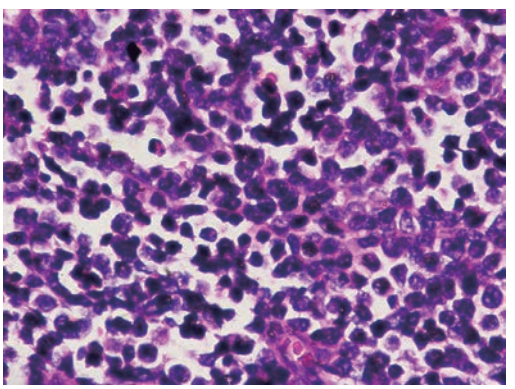

Fig. 2 Pathological examination of the gastric remnant mass showing diffuse proliferation of the small cells with scant cytoplasm and hyperchromatic nuclei.

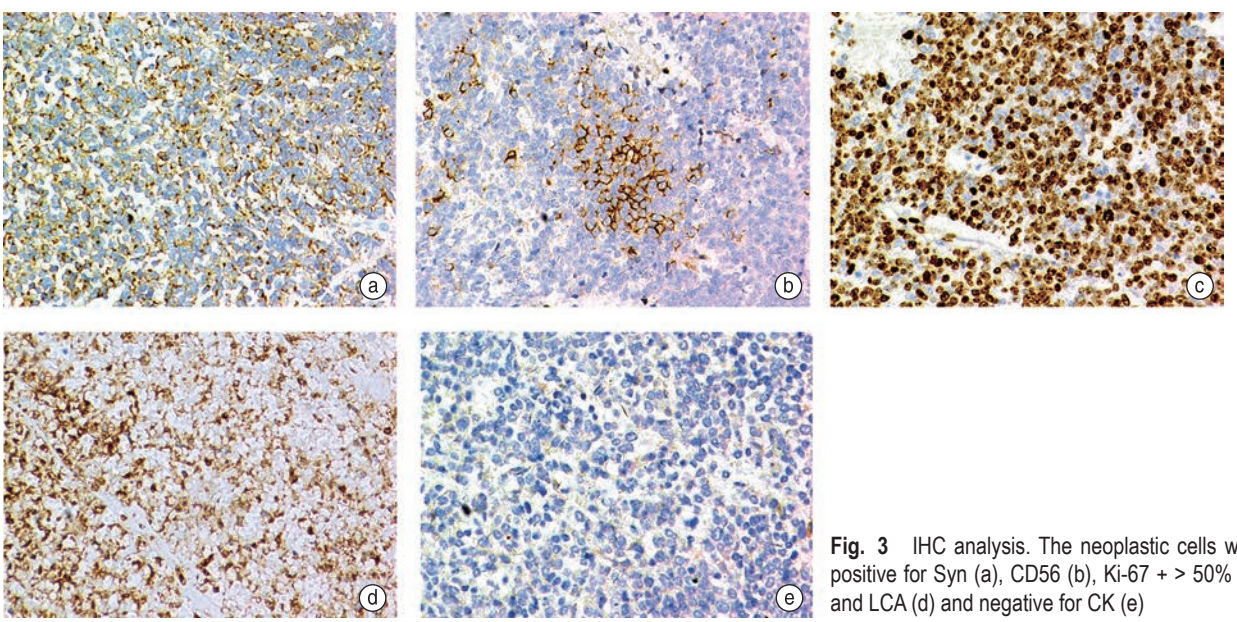

Fig. 3 IHC analysis. The neoplastic cells were positive for Syn (a), CD56 (b), Ki-67 + > 50% (c), and LCA (d) and negative for CK (e)

head, neck, and urinary tract and is rarely observed in the gastrointestinal tract [2]. Brenner [4] reported that SCC of the gastrointestinal tract mostly involved the esophagus (53%), followed by the colon (13%) and stomach (11%). Primary GSCC accounts for 0.1% of all gastric carcinoma cases [3] and was first described in 1976 by Matsusaka [5]. SCC of the gastric remnant is even less common.

GSCC may be either a pure or composite types. Pure-type GSCC is based on histologic specimens in which no other tumor types are identified, whereas composite-type GSCC consists of glandular and/or squamous differentiation along with SCC [2]. Moise [2] reported approximately equal numbers of cases of the two types. Matsui [6] reported that SCC originates from preexisting neuroectodermal cells, adenocarcinoma precursor cells, or pluripotent epithelial stem cells, which can result in dual or multiple differentiation such as a mixture of small neoplastic, squamous, and adenocarcinomatous cells. The microscopic features are frequently similar to those of other malignancies such as malignant lymphoma or undifferentiated carcinoma [7]. The histologic features of GSCC are similar to those of EPSCC, including features such as scanty cytoplasm and solid growth of small cells with hyperchromatic nuclei [3].

GSCC mostly occurs in men in their mid-sixties [7] who present with epigastric pain, nausea, anorexia, early satiety, and weight loss [2]. It is extremely difficult to diagnose GSCC before surgery [8]. Only 40% of patients with GSCC are diagnosed correctly [9]. Histological and IHC analyses are valuable for pathological diagnosis [2], including positive staining for neuron-specific enolase (NSE), chromogranin A (CGA), Grimelius, and Syn, which are reported to have high positivity rates in GSCC [8], with only 10%-20% of GSCC cases being negative for these tumor markers [2]. CEA staining is helpful to rule out adenocarcinoma [9]. CD56 markers can also be used to differentiate SCC from large cell carcinoma [10].

Our patient, who presented with epigastric pain, acid regurgitation and belching, was diagnosed based on histological and IHC staining. Pathology of the biopsy specimen showed SCC of the gastric remnant, with hyperchromatic nuclei and scant cytoplasm, whereas IHC analysis revealed neoplastic cells positive for Syn, LCA, CD56, and Ki-67 +>50%, similar to GSCC, as previously published. The diagnosis of SCC of the gastric remnant is similar to that of GSCC, although it is less reported. Therefore, to improve the accuracy of diagnosis, when SCC of the gastric remnant is morphologically suspected, additional IHC staining of CGA, Syn, NSE, Grimelius, and CD56 should be performed.

The standard treatment of GSCC remains unclear due to the rarity of this disease. Surgical treatment and intensive chemotherapy have been used alone or in combination with other treatments [11]. However, previous literature reported that GSCC was characterized by invasion and metastasis, which led to a poor prognosis [6]. Most patients with GSCC died within one year after diagnosis [9]. Matsui [5] reported a median GSCC survival time of less than 10 months. Most patients did not undergo chemotherapy in the postoperative period. However, Koide [1] reported a relapse-free survival period of more than 45 months following treatment with cisplatin (CDDP) and fluoropyrimidine S-1. Huang [11] reported a median survival of 48.5 months in patients who underwent curative surgery and adjuvant chemotherapy. Tanemura [8] reported PVP therapy, combining CDDP and etoposide (VP-16), to be effective against GSCC. Treatment of SCC of the gastric remnant is less reported. Our patient underwent total gastrectomy and adjuvant chemotherapy. Until now, he has been free of recurrence for 36 months. Long-term, regular follow-up is in progress.

In conclusion, SCC of the gastric remnant is an extremely rare malignant cancer characterized by invasion and metastasis [1]. Only few cases on SCC of the gastric remnant have been reported. Here, we report a patient with SCC of the gastric remnant to improve the diagnosis and treatment of SCC of the gastric remnant.

Acknowledgments

The authors thank all the patients and their families for participating in this research.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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Guideline Observation

Updates of the NCCN guidelines for non-small cell lung cancer

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Updates in version 4.2018 of the NCCN guidelines for non-small cell lung cancer from version 3.2018

NSCL-J 2 of 4

Adenocarcinoma, Large Cell, NSCLC NOS (PS 0–1)

Pembrolizumab/cisplatin/pemetrexed added as a first-line therapy option.

NSCL-J 4 of 4

Reference added: Gandhi L, Rodriguez-Abreu D, Gadgeel S, *et al*. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. N Engl J Med 2018 [published online April 16, 2018].

Updates in version 3.2018 of the NCCN guidelines for non-small cell lung cancer from version 2.2018

DIAG-3

Solitary part-solid nodule(s); sub-categories modified: Persistent and < 6 mm, delete "solid component"; Persistent and ≥ 6 mm, delete "solid component".

Sub-bullets added: If unchanged and solid component remains < 6 mm, annual CT for 5 y; If solid component ≥6 mm, consider PET/CT or biopsy.

Updates in Version 2.2018 of the NCCN Guidelines for Non-Small Cell Lung Cancer from Version 1.2018 include:

NSCL-4 and NSCL-6

Added Stage IIIA (T4, N0-1)

NSCL-17

Testing results clarification added: EGFR, ALK, ROS1, BRAF negative or unknown, PD-L1<50% or unknown

NSCL-J 2 of 4 and 3 of 4

Footnote ** added: Carboplatin-based regimens are often used for patients with comorbidities or those who cannot tolerate cisplatin.

MS-1

The Discussion section has been updated to reflect the changes in the algorithm.

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Guideline Observation

Updates of the NCCN guidelines for small cell lung cancer

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Updates in Version 2.2018 of the NCCN guidelines for small cell lung cancer from version 1.2018

The Discussion section has been updated to reflect the changes in the algorithm. (MS-1)

Updates in version 1.2018 of the NCCN guidelines for small cell lung cancer from version 3.2017

For consistency in imaging, statement was revised: "CT Chest / liver / adrenal" was replaced by "Chest / abdomen CT" with contrast.

Initial evaluation

Delete "Ca LDH", add "BUN"; Add "(skull base to mid-thigh)" to PET/CT scan, (if limited stage is suspected).

Footnote "b" for H & P was added: "See Signs and Symptoms of Small Cell Lung Cancer (SCL-A)" (Also for SCL-5).

Footnote "c" for pathology review was added: "See Principles of Pathologic Review (SCL-B)".

Additional workup

"During evaluation for surgery" was added to Pulmonary function tests (PFTs)

"(Consider biopsy if bone imaging is equivocal)" was added.

Adjuvant treatment

Clinical stage N+ separated into N1 and N2.

N1 adjuvant treatment option added: "Systemic therapy ± mediastinal RT (sequential or concurrent)"

N2 adjuvant treatment option added: "Systemic therapy + mediastinal RT (sequential or concurrent)".

Footnote "o"

Footnote "o" was modified: "For patients receiving adjuvant therapy, response assessment should occur only after completion of adjuvant therapy (SCL-5); do not repeat scans to assess response during adjuvant treatment."

Initial treatment of asymptomatic brain metastases

Statement was modified: "May administer the whole-brain RT after completion of systemic therapy".

Updates in version 1.2018 of the NCCN guidelines for small cell lung cancer from version 3.2017

Response assessment following initial therapy

Bullet 5 was modified: "Electrolytes, LFTs, BUN, creatinine". Deleted "Ca".

Adjuvant treatment; extensive disease

"PCI ± thoracic RT" revised to "Consider PCI ± thoracic RT".

Surveillance

Footnote "s" was added to heading: "See NCCN Guidelines for Survivorship".

Complete response or partial response

Limited stage

Statement was revised: "After completion of initial therapy" instaed of "After recovery from primary therapy".

Bullet 1 was revised: "Oncology follow-up visits every 3–4 mo during y 1–2, every 6 mo during y 3–5, then

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annually".

Bullet 1 revised: "At every visit: H&P, CT Chest/abdomen with contrast (delete liver/adrenal), bloodwork only as clinically indicated".

Bullet 2 was added: "If PCI not given, then MRI (preferred) or CT brain with contrast every 3–4 mo during y 1–2".

Extensive stage

Statement was added: "After completion of initial or subsequent therapy".

Bullet 1 was added: "Oncology follow-up visits every 2 mo during y 1, every 3–4 mo during y 2-3, then every 6 mo during years 4–5, then annually".

Bullet 1 revised: "At every visit: H&P, CT Chest/abdomen with contrast (delete liver/adrenal), bloodwork only as clinically indicated".

Bullet 2 was added: "If PCI not given, then MRI (preferred) or CT brain with contrast every 3–4 mo during y 1–2".

Footnote "u" for thoracic RT was revised: "Sequential radiotherapy to thorax in selected patients, especially with residual thoracic disease and low-bulk extrathoracic metastatic disease that has responded to systemic therapy." Deleted "complete response".

Stable disease

Limited stage and Extensive stage

Statement was revised: "After completion of initial therapy" delete "recovery from primary therapy".

Bullet 1 was revised: "Oncology follow-up visits every 3–4 mo during y 1–2, every 6 mo during y 3–5, then annually".

Statement was added: "After completion of initial or subsequent therapy".

Bullet 1 was added: "Oncology follow-up visits every 2 mo during y 1, every 3–4 mo during y 2–3, then every 6 mo during years 4–5, then annually".

SCL-6

Footnote "k," "See Principles of Supportive Care (SCL-D)" was added after all "Palliative symptom management" statements.

Footnote "v," "See Principles of Palliative Care (PAL-1)" was added after all "Palliative symptom management" statements.

For "PS 0-2," "or" was removed from between "Consider subsequent systemic therapy" and "Palliative symptom management, including localized RT to symptomatic sites".

(SCL-A) signs and symptoms of small cell lung cancer

A new section was added: "Signs and Symptoms of Small Cell Lung Cancer".

(SCL-B) principles of pathologic review

A new section was added: "Principles of Pathologic Review".

(SCL-C) principles of surgical resection

A footnote was removed: "Slotman B, Faivre-Finn C, Kramer G, *et al*. Prophylactic cranial irradiation in extensive small-cell lung cancer. N Engl J Med 2007; 357: 664-672."

(SCL-D) principles of supportive care

Syndrome of inappropriate antidiuretic hormone

Sub-bullet 5 was revised: "Vasopressin receptor inhibitors (conivaptan, tolvaptan) for refractory hyponatremia".

(SCL-E) principles of systemic therapy (1 of 3)

Extensive stage (maximum of 4–6 cycles)

Bullet 7 was revised: "Cisplatin 30 mg/m2 days 1, 8 and irinotecan 65 mg/m2 days 1, 8".

Footnote "†" was added: "If not used as original regimen, may be used as therapy for primary progressive disease."

Subsequent systemic therapy

Footnote "‡" was added: "Subsequent systemic therapy refers to second-line and beyond therapy."

Relapse ≤ 6 mo, PS 0-2: nivolumab ± ipilimumab

Reference "22" was added: "Hellmann MD, Ott PA, Zugazagoitia J, et al. First report of a randomized expansion cohort from CheckMate 032 [abstract]. J Clin Oncol 2017;35: Abstract 8503."

(SCL-E) principles of systemic therapy (2 of 3)

Limited-stage

Sub-bullet 1 was revised: "For patients receiving adjuvant therapy, response assessment should occur only after completion of adjuvant therapy; do not repeat scans to assess response during adjuvant treatment."

(SCL-F) principles of radiation therapy (1 of 3)

General Principles

Bullet 4 was revised: "Use of more advanced technologies is appropriate when needed to deliver adequate tumor doses while respecting normal tissue dose constraints. Such technologies include (but are not limited to) 4D-CT and/or PET/CT simulation, IMRT/VMAT, IGRT, and motion management strategies. IMRT is preferred over 3D conformal external-beam RT (CRT) on the basis of reduced toxicity in the setting of concurrent chemotherapy/RT. Quality assurance measures are essential and are covered in the NSCLC guidelines (see NSCL-C)."

Reference "1" was added: "Chun SG, Hu C, Choy H, *et al*. Impact of intensity-modulated radiation therapy

technique for locally advanced non-small-cell lung cancer: a secondary analysis of the NRG oncology RTOG 0617 randomized clinical trial. J Clin Oncol 2017; 35: 56–62."

Limited Stage

Bullet 5 was revised: "Dose and schedule: For limited-stage SCLC, the optimal dose and schedule of RT have not been established; 45 Gy in 3 weeks (1.5 Gy twice daily [BID]) is superior (category 1) to 45 Gy in 5 weeks (1.8 Gy daily). When BID fractionation is used, there should be at least a 6-hour inter-fraction interval to allow for repair of normal tissue. If using once-daily RT, higher doses of 60–70 Gy should be used. The current randomized trial CALGB 30610/RTOG 0538 is comparing the standard arm of 45 Gy (BID) in 3 weeks to 70 Gy in 7 weeks; accrual to an experimental concomitant boost arm has closed. The European CONVERT trial demonstrated comparable overall survival and toxicity between 45 Gy (BID) and 66 Gy (daily)."

Reference 20 was added: "Faivre-Finn C, Snee M, Ashcroft L, *et al* Concurrent once-daily versus twice-daily chemoradiotherapy in patients with limited-stage small-cell lung cancer (CONVERT): an open-label, phase 3, randomised, superiority trial. Lancet Oncol 2017; 18: 1116-1125."

Extensive stage

Bullet 1 modified: "Consolidative thoracic RT is beneficial for selected patients with extensive-stage SCLC with CR or good response to systemic therapy. Studies have demonstrated that consolidative thoracic RT up to definitive doses is well tolerated, results in fewer symptomatic chest recurrences, and improves long-term survival in some patients. The Dutch CREST randomized trial of modest-dose thoracic RT (30 Gy in 10 fractions), in patients with extensive stage SCLC that responded to systemic therapy demonstrated significantly improved 2-year overall survival and six-month PFS, although the protocol-defined primary endpoint of one-year overall survival was not significantly improved. Subsequent exploratory analysis found the benefit of consolidative thoracic RT is limited to the majority of patients who had residual thoracic disease after systemic therapy."

Bullet 2 was added: "Dosing and fractionation of consolidative thoracic RT should be individualized within the range of 30 Gy in 10 daily fractions to 60 Gy in 30 daily fractions, or equivalent regimens in this range."

Reference 24 was added: "Slotman BJ, van Tinteren H, Praag JO, *et al*. Radiotherapy for extensive stage small-cell lung cancer– Authors reply. Lancet 2015; 385: 1292–1293."

Prophylactic Cranial Irradiation (PCI)

Bullet 1 modified: "In patients with limited-stage SCLC who have a good response to initial therapy, PCI decreases brain metastases and increases overall survival (category 1).

In patients with extensive-stage SCLC that has responded to systemic therapy, PCI decreases brain metastases. A randomized trial conducted by the EORTC found improved overall survival with PCI. However, a Japanese randomized trial found that in patients who had no brain metastases on baseline MRI, PCI did not improve overall survival compared with routine surveillance MRI and treatment of asymptomatic brain metastases upon detection. In patients not receiving PCI, surveillance for metastases by brain imaging should be considered performed."

Bullet 5 was added: "When administering PCI, consider adding memantine during and after RT, which has been shown to decrease neurocognitive impairment following whole brain radiation therapy (WBRT) for brain metastases."

Reference 28 was updated: "Takahashi T, Yamanaka T,Takashi S et al. Prophylactic cranial irradiation versus observation in patients with extensive-disease small-cell lung cancer: a multicentre, randomised, open-label, phase 3 trial. Lancet Oncol 2017; 18: 663–671."

Reference 31 was added: "Brown PD, Pugh S, Laack NN, *et al*. Memantine for the prevention of cognitive dysfunction in patients receiving whole-brain radiotherapy: a randomized, double-blind, placebo-controlled trial. Neuro Oncol 2013; 10: 1429–1437."

Brain Metastases

Bullet 1 modified: "Brain metastases should be treated with WBRT rather than stereotactic radiotherapy/ radiosurgery (SRT/SRS) alone, because these patients tend to develop multiple CNS metastases. In patients who develop brain metastases after PCI, repeat WBRT may be considered in carefully selected patients. SRS, is preferred if feasible, especially if there has been a long-time interval from initial diagnosis to occurrence of brain metastases and there is no uncontrolled extracranial disease."

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