Original Article

Expressions of Thymidylate Synthase, Thymidine Phosphorylase, Class III β-tubulin, and Excision Repair Cross-complementing Group 1 Predict Response in Advanced Gastric Cancer Patients Receiving Capecitabine Plus Paclitaxel or Cisplatin

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ABSTRACT

Objective: To evaluate the role of class III β-tubulin (TUBB3), thymidylate synthase (TS), thymidine phosphorylase (TP), and excision repair cross-complementing group 1 (ERCC1) in clinical outcome of advanced gastric cancer patients receiving capecitabine plus paclitaxel or cisplatin.

Methods: The clinical data and tumor specimens from 57 advanced gastric cancer patients receiving first-line capecitabine plus paclitaxel (cohort 1, n=36) and capecitabine plus cisplatin (cohort 2, n=21) were retrospectively collected, and TUBB3, TS, TP, and ERCC1 expressions were detected by real-time quantitative PCR. The associations between expressions of biomarkers and response or survival were analyzed statistically.

Results: The median age of 57 patients was 57 years (range: 27–75 years) with 38 males and 19 females. Of all patients, the response rates of patients with high TP, low TP and high TS, low TS expressions were 57.1%, 27.6% (P=0.024), and 55.2%, 28.6% (P=0.042), respectively. Among cohort 1, the response rates and median overall survivals of patients with low and high TUBB3 expressions were 61.1% vs. 33.3% (P=0.095) and 13.8 months vs. 6.6 months (P=0.019), respectively; the response rate (87.5%) of patients with low TUBB3 and high TP expressions was higher than that (14.3%) of patients with high TUBB3 and low TP expressions (P=0.01). Among cohort 2, the response rates of patients with low ERCC1 and high ERCC1 expressions were 45.5% and 20.0% respectively (P=0.361).

Conclusion: TUBB3, TS and TP expressions could predict the response of advanced gastric cancer patients receiving capecitabine-based and paclitaxel-based chemotherapy. These results will be further confirmed in future large samples.

Key words: Advanced gastric cancer; TS/TP/TUBB3/ERCC1; Capecitabine; Paclitaxel; Cisplatin

INTRODUCTION

Capecitabine combined with cisplatin or paclitaxel has been an effective combination regimen used in patients with advanced gastric cancer. These regimens have increased response rate to over 40%, but have not prolonged the median overall survival time. Early studies showed that more than half of gastric cancer patients do not benefit from capecitabine-based chemotherapy[1-4]. Therefore, a great of effort has been made to identify patients who are most likely to benefit from these regimens.

A growing body of evidence suggests that the intratumor gene expression of drug-metabolizing enzymes, DNA repair enzymes, or angiogenic enzymes may have important implications for anticancer drug efficacy[5-7]. Both thymidylate synthase (TS) and thymidine phosphorylase (TP) are two key enzymes involved in metabolic pathway of fluoropyrimdines. As the target of taxanes, class III β-tubulin (TUBB3) over-expression may have clinical relevance in the efficacy of taxanes therapy. The product of excision repair cross-complementing group 1 (ERCC1) gene is an essential member of the nucleotide excision repair (NER) pathway which is involved in the repair of DNA

double-strand breaks. ERCC1 gene expression may have associations with clinical outcome to platinum-based chemotherapy. Therefore, these biomarkers may be potential candidates for predicting chemotherapy efficacy in gastric cancer patients treated with capepiciabine and paclitaxel or cisplatin.

So far, no standard chemotherapy regimen has been established for advanced gastric cancer treatment. Thus, it is critical to identify biomarker(s) associated with clinical outcomes that may improve treatment success and tailor the chemotherapy regimen. This study was designed to identify potential predictors that may influence response to chemotherapy and survival in patients with advanced gastric cancer.

MATERIALS AND METHODS

Patients Eligibility Criteria

All patients in this study had histologically confirmed metastatic adenocarcinoma of stomach and at least one measurable lesion according to the Response Evaluation Criteria in Solid Tumors (RECIST). These patients were treated by capecitabine/paclitaxel or capecitabine/cisplatin chemotherapy in the Department of Gastrointestinal Oncology, Beijing Cancer Hospital and had completed at least two cycles of study treatment. No patients with prior chemotherapy except for neoadjuvant or adjuvant chemotherapy treatment completed 12 months before the study were enrolled. All patients underwent endoscopic biopsy from primary stomach or vacuum-associated core needle biopsy from metastatic lesion before chemotherapy. The tissue samples were collected retrospectively from patients who met these criteria. Chemotherapeutic response was clinically evaluated by measuring the change in tumor size according to the standard RECISE as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). CR and PR were defined as responders. Stable disease and progressive disease were defined as nonresponders. The time to progression (TTP) and overall survival (OS) were calculated from the date therapy was started, to the date of disease progression and death, respectively. Written informed consent was obtained before treatment and evaluation of tumor samples. The use of all patient material was approved by our institutional review board.

Treatment Protocols

The following first-line chemotherapy regimens were administered to the patients in our study: capecitabine plus paclitaxel or capecitabine plus cisplatin; capecitabine was orally administered at a dosage of 1,000 mg/m^2 twice daily from the evening of day 1 until the morning of day 15 within each 3 weeks cycle. Paclitaxel was given at a dosage of 80 mg/m^2 as a 180-min iv infusion on days 1 and 8 of each cycle. Cisplatin was given at a dosage of 80 mg/m^2 as a 120-minute iv infusion on day 1 of each cycle. Treatment was continued until disease progression, unacceptable toxicity, or patient/physician decision.

RNA Isolation and cDNA Synthesis

Total RNA from tumor specimens was extracted from formalin-fixed, paraffin-embedded tissues using 10-μm sections. For histologic diagnosis, representative sections were stained with haematoxylin and eosin by standard methods. Macroscopic dissection was used to ensure the extraction of RNA from defined areas with at least 60% tumor cells. Total RNA was extracted using HighPure RNA Paraffin Kit (Roche Diagnostics GmbH, Mannheim, Germany) in accordance with the supplied protocol. Total RNA were reversetranscribed for single-strand cDNA using Exscript™ RT reagent Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacture's protocol (Applied Biosystems). Reverse transcription reaction was performed at 25°C for 10 min, 37°C for 120 min, followed by heating at 85°C for 5 min.

Reverse Transcription-PCR Quantification of mRNA

The relative quantitation of cDNA for TP, TS, TUBB3, ERCC1 and an internal reference gene (β-actin) was done using a fluorescence-based, real-time detection method (Rothe Sequence Detection System; SYBR GREEN I; Applied Biosystems), according to the supplier's instructions. The primer sequences are shown in Table 1.

Table 1. Sequences of primers

	Forward	Reverse
TP	5'-TGGCTGCAAGGTGCCAATG-3'	5'-AGCACTTGCATCTGCTCTGG-3'
TS	5'-GCCTCGGTGTGCCTTTCA-3'	5'-CCCGTGATGTGCGCAAT-3'
TUBB3	5'-GGCCAAGTTCTGGGAAGTCAT-3'	5'-GAGGCCTCGTTGTAGTAGACG-3'
ERCC1	5'-GGGAATTTGGCGACTGTAATTC-3'	5'-GCGGAGGCTGAGGAACAG-3'
β-actin	5'-TGAGCGCGGCTACAGCTT-3'	5'-TCCTTAATGTCACGCACGATTT-3'

The PCR reaction mixture consisted of 600 nmol/L each primer, 2.5 U Ampli Taq Gold polymerase, 200 nmol/L each dATP, dCTP, dGTP and 400 μmol/L dUTP, 5.5 mmol/L MgCl$_2$, and 1 μl Taqman Buffer A containing a reference dye, to a final volume of 25 μl (all reagents were purchased from Applied Biosystems). Cycling conditions were 50°C for 2 min, 95°C for 10 min, followed by 46 cycles at 95°C for 15 s and 60°C for 1 min.

Relative gene expression quantification was calculated according to the comparative Ct method by