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Original Article

Genomic predictors of chemotherapy efficacy in advanced or recurrent gastric cancer in the GC0301/TOP002 phase III clinical trial

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ABSTRACT

Recent gastric cancer clinical trials have aimed to establish the efficacy of combination therapy over monotherapy, however, the role for genomic biomarkers in these trials has remained largely unexplored. Here, using the NanoString expression platform, we analyzed 105 gastric tumors from a randomized phase III Japanese clinical trial (GC0301/TOP002) testing the efficacy of irinotecan plus S-1(IRI-S) versus S-1 therapy. We found that previously established proliferative subtype signatures, were associated with older patients (>65 years) and liver metastasis while mesenchymal subtype signatures were associated with younger patients (≤65 years) and peritoneal metastasis. Genes associated with tumor microenvironment (*CD4*, *CD14*, *ADAMTS1*, *CCL5*, *CXCL12*, *CCL19*), therapeutic implications (*DPYD*) and oncogenic signaling (*Wnt5A*, *PTRF*) were significantly associated with patient age, histology, tumor status, measurable lesions and metastasis. We identified *Wnt5A* downregulation as a candidate predictor of improved progression free survival (>8 weeks) in S-1 but not in IRI-S treatment. Although statistical significance was not achieved, mesenchymal subtype showed a trend for treatment interaction with IRI-S for efficacy. These findings highlight promising genomic markers that could be useful predictors of chemotherapy efficacy for better prognosis and survival outcome in gastric cancer.

Introduction

Gastric cancer (GC) prevalence and mortality is more common in Asia compared to the West [1]. Despite great advances in diagnosis and treatment, a poor outcome from advanced and unresectable GC is still evident. Several randomized controlled clinical trials of various chemotherapeutic regimens have been performed in patients with advanced GC but with little significant improvement [2–6]. The more recent ones such as the REGARD trial [7], RAINBOW trial [8] the GRANITE-I [9] studies also did not demonstrate much significant improvement in OS or PFS compared with best supportive care.

Although a global standard regimen has not yet been defined, 5-FU plus a platinum is preferred worldwide. The 5-FU is given

Abbreviations: 5FU, 5 Fluorouracil; S-1, tegafur/gimeracil/oteracil; IRI-S, Irinotecan plus S1; TS, thymidylate synthase; DPYD, dihydropyrimidine dehydrogenase; *ERCC1*, excision repair cross -complementation group 1; *GAPDH*, Glyceraldehyde-3-Phosphate Dehydrogenase; *TBP*, TATA-Box Binding Protein; *ACTB*, Actin Beta; *RPL29*, Ribosomal Protein L29; *GUSB*, Glucuronidase Beta; PFS, Progression-free survival; OS, Overall survival; *CD14*, CD14 molecule; ECOG-PS, the Eastern Cooperative Oncology Group performance status; *Wnt5A*, Wnt Family Member 5A; *PTRF*, Polymerase I And Transcript Release Factor; *CCL5*, C-C Motif Chemokine Ligand 5; *CXCL12*, C-X-C Motif Chemokine Ligand 12; *CCL19*, C-C Motif Chemokine Ligand 19; *CXCR4*, C-X-C Motif Chemokine Receptor 4.

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intravenously or substituted with its oral derivatives (pro-drugs), S-1 or capecitabine to the GC patients. S-1 is commonly used in Asia due to a mild toxicity compared with Western countries. Phase I/II studies of S-1 have shown responses of more than 50% in patients with advanced GC [2,10]. Patients with no response to first-line treatment with S-1 often receive a taxane or irinotecan (IRI) alone as second-line treatment. Although second-line treatment have been shown to prolong survival in these patients, a combination therapy is suggested to work better, showing a higher response rate from IRI-S treatment compared to IRI alone treatment [11,12]. However, a superior efficacy of IRI-S over S-1 alone treatment could not be shown in the multicenter, randomized phase III clinical trials in Japan (JACCRO GC-05 and GC0301/TOP-002) [6,13]. These studies have created a need for molecular evaluation of the gastric tumors which may help to identify biomarkers and stratify the patients according to the biomarkers for predicting response to therapy.

In the era of biomarker-directed therapy, evaluating expression of gene signatures or multiple genes associated with patient clinical response and outcome in randomized clinical trials is an important challenge in precision oncology. The expression of signature subtype specific studies that involve comparison of the gene expression in groups of patient tumors defined by histopathological features have been suggested to identify differentially expressed genes between the different subtypes. This could improve our understanding of the biological processes and also identify the predictors of prognosis and response to chemotherapy. This has been highlighted by several groups including The Cancer Genome Atlas [14] and the Asian Cancer Research Group (ACRG) [15]. Taking this into consideration, we hypothesized that the expression of gene signatures or multiple genes associated with patient clinical response and outcome in randomized GC clinical trial (GC301/TOP-002) could play a role in assessing the treatment effects.

New genomic technologies such as the NanoString nCounter system have been used in several clinical trial studies for prognostic and predictive markers [16–18]. Here, we used this system to further expand the GC301/TOP-002 trial sub study and investigated the expression of a panel of 500 GC relevant genes from the RNA samples. We aimed to perform a comprehensive analysis on a NanoString platform and examined the relationship between GC relevant genes and signature subtypes with patients' treatment response and survival outcome and their interaction with S-1 monotherapy and IRI-S combined therapy.

Materials and methods

Patient population

A total of 326 patients (Fig. 1) with advanced or recurrent GC were treated in the multicenter, randomized phase III clinical trial in Japan (GC0301/TOP-002) that compared the efficacy and safety of IRI-S combination with S-1 monotherapy [6]. The inclusion criteria into the trial were histologically and cytologically confirmed unresectable or recurrent gastric adenocarcinoma; oral food intake possible, age between 20 and 75 years, no prior radiotherapy or chemotherapy, expected survival for >12 weeks, ECOG performance status of 0–2 and adequate major organ function before chemotherapy. Written informed consent was obtained before registration and the opportunity to refuse to provide tumor samples was open to the public according to the Japanese Ethical Guidelines for Clinical Studies. The study was approved by the institutional review board of Japanese Gastric Cancer Association and each participating hospital. The patients (S-1 monotherapy, n = 162; IRI-S, n = 164) received oral S-1 (80mg/m2 daily for 28 days every 6 weeks) or oral S-1 (80mg/m2 daily for 21 days every 5 weeks) plus IRI (80mg/m2 by intravenous infusion on days 1 and 15 every 5 weeks) respectively. The median overall survival with IRI-S versus S-1 was 12.8 versus 10.5 months, respectively (HR 0.856, P = 0.233). In the post-hoc subset analyses, IRI-S was significantly more effective than S-1 for patients with diffuse type histology and for those with an ECOG-PS 1 or 2, suggesting that some group of patients might benefit from irinotecan doublets [6].

The gastric cancer tissues (n = 105) were obtained from 50 biopsies and 55 surgically resected specimens for patients who consented for their specimens for the current study. The tissues were formalin-fixed paraffin embedded and sectioned in Hitachi Chemical Company Ltd, Japan. Tumor cells were carefully laser microdissected or manually macro dissected and the RNA isolated. The RNA samples were also used previously for another sub study to measure the expression levels of five specific genes related to DNA repair and 5-FU metabolic pathway (*TS*, *DYPD*, topoisomerase I, *ERCC1*, thymidine phosphorylase), A low TS, low ERCC1 and high thymidine phosphorylase were reported to be associated with better prognosis for IRI-S versus S-1 [19].

NanoString assay

The RNA samples (n = 105) for the current study were quantified using Nanodrop 1000 instrument, in Hitachi Chemical Company Ltd. Japan and their integrity was assessed by Agilent 2100 bioanalyzer (Agilent Technologies) in Duke-NUS Medical School, Singapore. A NanoString panel comprising of 500 genes was designed including 5 housekeeping genes (*GAPDH*, *TBP*, *ACTB*, *RPL29* and *GUSB*). The 495 target genes consisted of three GC signatures (n = 258) (Supplementary Table 1) and single genes (n = 237) related to GC oncogenic signaling pathways (Supplementary Table 2).

NanoString probes and 100 ng of patients' RNA were hybridized overnight at 65 °C according to the manufacturer's protocol (NanoString Technologies Inc.). Raw expression data representing the number of transcripts were counted using a NanoString nCounter Digital Analyzer. The data was then normalized using NanoString nSolver Analysis software. Normalization factor was calculated relative to the expression levels of housekeeping genes as described previously [16] and the data was log2 transformed for further analysis.

Statistical analysis

The normalized log2 transformed mRNA expression data was analyzed by unsupervised hierarchical clustering using Cluster version 3.0 and Java Tree view. The gastric tumors were mapped according to the previously established subtypes in each signature and correlated with the patient clinico-pathological characteristics, response and survival. Heat maps showing high and low gene expression levels in the signature subtypes were generated and patient samples were categorized based on these expression pattern clusters. Fisher's exact test was used to evaluate associations between gene expression patterns or signature clusters and patient clinicopathological characteristics. Single genes were analyzed based on their median expression levels as cut-off points.

To assess the interaction effects between treatment arm and biomarkers on progression-free survival (PFS) and overall survival (OS), we used Cox proportional hazard models adjusted for age, gender, ECOG-PS, Lauren's histology, tumor status, measurable lesion (Table 1) with or without, interaction terms between treatment arm and biomarkers. Patient response without measurable lesions (non-target lesions) was evaluated by RECIST version 1.0. Complete response (CR) was defined as disappearance of all non-target lesions and normalization of tumor marker level. Incomplete response or Stable disease (SD) was defined as persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits and Progressive disease (PD) was defined as the appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. Patients response criteria with target or measurable lesions included the above response groups and also partial response (PR) with a 30% decrease in the sum of the largest diameter of target lesions taking as reference the baseline sum of the largest diameter.

Accordingly, for statistical analysis, we grouped the partial and stable response as “good” and progressive and not evaluable as “poor”. Overall survival time was defined as the interval from randomization to the date of death (patients who remained alive at the final follow-up were censored at that time). Also PFS was calculated from randomization to the first objective evidence of disease progression or death from any cause. All tests were two-sided at significance level $p < 0.05$. The p-values of interactions between treatments and biomarkers were calculated using the likelihood ratio tests. The statistical analysis was performed using IBM-SPSS Statistics version 22.0 for Windows (SPSS Inc., Chicago, IL, USA) and SAS statistical package version 9.3 (SAS Institute Inc., Cary, NC, USA).

Results

Gene signature subtype expression analysis

A total of 326 patients were enrolled in the GC301/TOP-002 trial and RNA for NanoString profiling was performed for 105 patients (32.2%) (Fig. 1). The baseline demographic and clinical characteristics of the subjects (Table 1) were similar to the results of the GC0301/TOP-002 trial [6]. Heat maps from NanoString data were generated based on three previously reported GC molecular subtypes [20–22]. Hierarchical clustering was performed to validate the existing data and identify the predictive biomarkers with