Predictive biomarkers for 5-fluorouracil and oxaliplatin-based chemotherapy in gastric cancers via profiling of patient-derived xenografts

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Gastric cancer (GC) is commonly treated by chemotherapy using 5-fluorouracil (5-FU) derivatives and platinum combination, but predictive biomarker remains lacking. We develop patient-derived xenografts (PDXs) from 31 GC patients and treat with a combination of 5-FU and oxaliplatin, to determine biomarkers associated with responsiveness. When the PDXs are defined as either responders or non-responders according to tumor volume change after treatment, the responsiveness of PDXs is significantly consistent with the respective clinical outcomes of the patients. An integrative genomic and transcriptomic analysis of PDXs reveals that pathways associated with cell-to-cell and cell-to-extracellular matrix interactions enriched among the non-responders in both cancer cells and the tumor microenvironment (TME). We develop a 30-gene prediction model to determine the responsiveness to 5-FU and oxaliplatin-based chemotherapy and confirm the significant poor survival outcomes among cases classified as non-responder-like in three independent GC cohorts. Our study may inform clinical decision-making when designing treatment strategies.
**Results**

**Estimation of responsiveness to 5-FU and oxaliplatin-based chemotherapy using GC PDX models.** To investigate potential predictive factors associated with chemotherapy responsiveness in GCs, we established a GC PDX cohort, consisting of 32 PDX cases from 31 GC patients, and performed in vivo screening for 5-FU and oxaliplatin-based chemotherapy (Fig. 1a and Supplementary Table 1, age: 40–86 years, sex: 25 males and 6 females). PDX tumors retained drug responsiveness in our PDX cohort applicable to Food and Drug Administration-approved drugs and standard care (OncoKB level 1 and 2; http://www.oncokb.org) were maintained in all of the patient and PDX pairs (Supplementary Fig. 1 and Supplementary Data 1). Clonal structures, estimated using the PyClone algorithm, were well conserved between patients and PDX tumors (Supplementary Fig. 2a, b). Because FOLFOX is a widely used standard chemotherapy regimen among GC patients, we treated the GC PDX models with 5-FU + oxaliplatin. The PDX models were classified into 13 responders (Rs), 11 non-responders (NRs), and 8 cases with questionable responsiveness (Fig. 1a, b). Rs were classified by comprehensive evaluation in that it reflects the tumor volumes before and after treatment with both drug- and vehicle-treated samples using the following criteria: (1) drug treatment significantly inhibited tumor growth (two-way analysis of variance (ANOVA) \( P < 0.0001 \); Supplementary Fig. 3a–c and Supplementary Table 2); and (2) the percent tumor growth inhibition (TGI (%); described in “Methods”) by drug treatment at end point was \( >60\% \) (Fig. 1b and Supplementary Table 2). The average TGI was 81.8 ± 22.9% in Rs and NRs, respectively, with a significant difference (\( U \) test \( P < 0.0001 \)). However, tumor growth rates between the R and NR groups did not differ significantly during the initial xenotransplantation stage (Passage 0) (Supplementary Fig. 4a) or in vehicle-treated mice (Passage 2; Supplementary Fig. 4b), suggesting that tumor growth rate had little effect on the responsiveness classification.

Next, we compared responsiveness to 5-FU and oxaliplatin in PDX models with clinical responsiveness in matched patients. Among 10 patients who were treated with a comparable regimen, 3 of the 4 patients whose PDX models were classified into the R group showed clinically relevant responsiveness to 5-FU and platinum-based regimens (median progression-free survival time >46 months; Fig. 1c, d). Representative, patient #3 received 54 cycles of XELOX (capecitabine plus oxaliplatin) treatment and showed no signs of disease progression for 51 weeks (Fig. 1d). In contrast, all 6 patients whose PDX models were classified as NR exhibited treatment resistance to 5-FU + platinum-based regimens, resulting in relapse or tumor progression (median progression-free or disease-free survival time of 5 months, ranging from 1 to 25 months; Fig. 1c, d). Drug responsiveness in the PDX models was significantly concordant with recurrence in matched patients (\( P = 0.033 \), Chi-square test), and patients classified as NR, based on the PDX response, showed poorer prognosis than patients classified as R, based on progression-free survival (\( P = 0.0212 \), log-rank test; Fig. 1e). These data suggested that responsiveness to 5-FU and oxaliplatin-based chemotherapy could be reliably predicted according to the PDX model criteria, anticipating the clinical responsiveness in patients.

**Investigation of previously reported factors associated with 5-FU responsiveness in our PDX cohort.** First, we compared clinical characteristics between R and NR patients, classified based on the PDX model response. Several clinical parameters, including gender, age, World Health Organization and Lauren classifications, tumor location, and tumor stage [Tumor, Node, Metastasis (TNM)], were not associated with responsiveness to 5-FU and oxaliplatin (Supplementary Table 3). Additionally, based on molecular profiling, TCGA molecular GC subtypes were not associated with drug responsiveness (\( P = 0.464 \); Supplementary Table 4).

Previous studies suggested that genes in the one-carbon metabolism pathway, microsatellite instability, and p53 status were associated with the 5-FU clinical response, for many cancer types. The one-carbon metabolism pathway includes methylenetetrahydrofolate reductase (MTHFR),...
methionine synthase (MTR), methionine synthase reductase (MTRR), and thymidylate synthase (TYMS)\textsuperscript{10}. Single-nucleotide polymorphisms (SNPs) and gene expression levels were suggested to serve as predictive markers for 5-FU-based chemotherapy responsiveness\textsuperscript{10}. However, no differences were observed in germline and somatic mutation status (Supplementary Fig. 5a, b) or one-carbon metabolism-associated gene expression levels (Supplementary Fig. 5c) between the R and NR groups in our study.

Microsatellite instability results from the inactivation of mismatch repair (MMR) pathways and the benefit from FOLFOX chemotherapy with MSI tumors remains controversial\textsuperscript{11,13}. When we examined MMR pathway genes, recurrent somatic mutations in these genes were not associated with 5-FU + oxaliplatin treatment responsiveness among our PDX cohort (Supplementary Fig. 6a). The expression levels of MLH1, which is one of the essential MMR genes, was not significantly different between the R and NR groups (Supplementary Fig. 6b). However, mutation
burden was highly correlated with defective MMR signature, and MLH1 expression showed significant anti-correlation with mutation burden and defective MMR signature (Supplementary Fig. 6c).

Functional p53 protein status has been suggested to affect 5-FU-based chemotherapy sensitivity in several cancer types. Although not significantly, the somatic mutations frequencies in TP53 increased in the NR group relative to the R group (38% for the R group and 64% for the NR group; Supplementary Fig. 7a, b), and the average p53 activity score was higher in the R group (0.19) than in the NR group (−0.11, Supplementary Fig. 7c). In addition, NRs with no TP53 mutation also showed low p53 activity (group average: −0.13; Supplementary Fig. 7c), suggesting defective p53 function among the NR group. Although various factors associated with the MMR process and the p53 pathway were associated with the R or NR groups, previously suggested candidate predictive factors for 5-FU-based treatment did not sufficiently explain drug responsiveness in our GC PDX cohort, suggesting the need for additional treatment responsiveness biomarkers to be developed.

Comprehensive genomic characterization of Rs and NRs to 5-FU and oxaliplatin-based chemotherapy. To identify novel genomic features that separate the R and NR groups, we analyzed the genomic profiles of PDX tumors, using whole-exome sequencing (WES). Difference in the frequency of MSI-high samples (6/13 in Rs, 3/11 in NRs, P = 0.597) and tumor mutation burden (TMB; P = 0.254, when the blood DNA unmatched samples were excluded) were not significant between groups (Fig. 2a). In GC-associated cancer genes, suggested by the TCGA report, no genes with significantly different mutation frequencies were identified between the R and NR groups (Fig. 2a).

When comparing whole-exome mutation profiles between the R and NR groups, we identified 11 exclusively mutated cancer-associated genes, including KIAA1549 (4/13), BRD3 (3/13), ZMYM2 (3/13), PDGFRα (3/13), ICE1 (3/13), and TRIM33 (3/13), in the R group, and BCL9 (4/11), HEY1 (3/11), FOXO3 (3/11), NCOA2 (3/11), and DDR2 (2/11), in the NR group (Fisher’s exact test, P < 0.1, Fig. 2a). The functional changes and clinical implications of these mutations remain to be further clarified.

Metabolic pathways define Rs, and cell-to-cell interactions define NRs to 5-FU and oxaliplatin-based chemotherapy. We investigated the transcriptomic signatures of the R and NR groups by analyzing RNA sequencing data from PDX tumors. R and NR tumors were not easily discernible according to overall expression pattern due to mixed heterogeneous expression patterns, without distinct clustering patterns (Supplementary Fig. 9a). This indicates that drug responsiveness is not largely reflected by general gene expression patterns; instead, specific markers need to be examined through comparisons of the R and NR groups.

Gene set enrichment analysis (GSEA; Fig. 3a) and pathway analyses, based on differentially expressed genes (DEGs; Supplementary Fig. 9b, c) in the PDX transcriptome, revealed the enrichment of common gene sets associated with cancer.
metabolism, including "REACTOME: mitochondrial fatty acid beta-oxidation," "KEGG: metabolism of xenobiotics by cytochrome P450," and "KEGG: biosynthesis of unsaturated fatty acids," in the R group (Fig. 3a and Supplementary Fig. 9b). In contrast, the NR group was enriched in gene sets associated with cell-to-cell and cell-to-extracellular matrix (ECM) interactions, including "REACTOME: MET activates PTK2 signaling," "HALLMARK: Wnt/β-catenin signaling," "KEGG: ECM-receptor interaction," and "HALLMARK: angiogenesis" (Fig. 3a and Supplementary Fig. 9c).

To identify more robust predictive tumor expression markers associated with responsiveness to 5-FU and oxaliplatin-based
chemotherapy, we also analyzed the transcriptomes of matched human cancer tissues, and DEGs that were regulated in both patient and PDX tumors were compared between groups. A total of 40 upregulated genes shared between patient and PDX tumors were identified for the R group (log2 fold-change ≥ 1.5, P < 0.1), including MDM2, TMPRSS2, and POUSRF51 (Fig. 3b). In contrast, 83 genes, including FGFR1, PDGFRα, MLLT11, and COL1A1, were upregulated in the NR group for both patient and PDX tumors (log2 fold-change ≤ -1.5, P < 0.1; Fig. 3b). A complete list of 123 DEGs is shown in Supplementary Data 2. Data integration between both exome and transcriptome analyses revealed fewer TP53 mutations and increased p53 activity, based on increased p53 target gene (MDM2 and CDKN1A) expression17,18, in the R group (Fig. 3b), suggesting that intact p53 signaling likely enhances responsiveness to 5-FU and oxaliplatin-based chemotherapy. Protein–protein interaction (PPI) networks and the functional enrichment analysis of the 83 commonly upregulated genes between patient and PDX tumors in the NR group showed the significant functional enrichment of “ECM–receptor interaction,” “Focal adhesion,” and “PI3K-Akt signaling pathway” gene sets (Fig. 3c). Of the 83 upregulated gene in the NR group, 19 genes were previously reported to be upregulated in EMT19 and 23 genes were associated with mesenchymal phenotype of GC20, and many of these genes were subtype marker genes in previous studies (Supplementary Fig. 10a). In addition, EMT marker genes such as SNAI1, MMP9, and FN1 showed significantly higher expression in the NR group (Supplementary Fig. 10b). These data emphasize the importance of p53 signaling and ECM interaction pathways for resistance to 5-FU and oxaliplatin-based chemotherapy.

Tumor microenvironment (TME) reflects cancer cell characteristics based on responsiveness to 5-FU and oxaliplatin-based chemotherapy. Because ECM–receptor interaction pathway activation was identified in the NR group of our PDX cohort (Fig. 3a, c), we further investigated the TME composition. In PDX models, TME components, including immune and stromal cells and non-cellular matrix proteins, are usually replaced by cells and matrix components of mouse origin21,22. Therefore, we analyzed mouse-originating RNA reads from the WTS analysis as representative of the TME23.

GSEA analysis of the TME, using mouse-originating sequenced reads from PDX models, revealed the highly enhanced metabolic features of the R group, including “Fatty acid metabolism” and “Xenobiotic metabolism” (Fig. 4a and Supplementary Fig. 11a), consistent with the patterns observed for human cancer cells. In addition, gene sets associated with cell-to-cell and cell-to-ECM interactions, such as “Transforming growth factor-β (TGF-β) signaling,” “Angiogenesis,” and “Wnt/β-Catenin signaling,” were enriched in the NR group (Fig. 4a and Supplementary Fig. 11a), consistent with the GSEA results from human cancer cells. Several TGF-β signaling pathway genes, such as Eng24 and Jag225, were upregulated in the NR group tumors (Fig. 4b), and Eng and Jag2 genes were also reported to be involved in the regulation of endothelial cell migration and endothelium development26,27. Comparing TME DEGs between groups for both patient (from the whole transcriptome) and PDX models (from mouse reads), we identified 27 and 23 genes enriched in the R and NR groups, respectively (Fig. 4c). Network analysis, using the STRING database for 23 TME-associated genes in the NR group, showed the functional enrichment of genes regulating angiogenesis, including Cdh5, Tie1, and Dll4 (Fig. 4d). These data suggested that the upregulation of metabolism-related pathways, in both tumor cells and the TME, cooperate to induce a favorable response to 5-FU and oxaliplatin-based chemotherapy, whereas cell-to-cell or cell-to-ECM interactions and angiogenesis were associated with an unfavorable response to 5-FU and oxaliplatin-based chemotherapy.

We also investigated the TME cellular composition, using transcriptome data for both patient and PDX tumors. The stromal scores of patient tumors were slightly higher in the NR group than in the R group (P = 0.09), and several cell types, such as endothelial cells (Mann–Whitney U test, P = 0.09), interstitial dendritic cells (Mann–Whitney U test, P = 0.09), and fibroblasts (Mann–Whitney U test, P = 0.14), were enriched in patient tumors from the NR group (Fig. 4e and Supplementary Fig. 11b). The enrichment of endothelial cells in patient tumors during cellular composition analyses was compatible with the increased expression of angiogenesis-related gene sets observed in the TME transcriptome analyses (Fig. 4d). The estimated proportion of endothelial cells in the PDX TME were higher in the NR group than in the R group, although this difference was not significant (Fig. 4e and Supplementary Fig. 11c). In addition, when we compared marker gene expression for TME components (leukocytes and cancer-associated fibroblasts) among PDX tumors, genes associated with cancer-associated fibroblasts were expressed at higher levels in the NR group than in the R group (Supplementary Fig. 11d). These data suggested that stromal cells in the TME, such as endothelial cells and fibroblasts, and their interactions with cancer cells play important roles in responsiveness to 5-FU and oxaliplatin-based chemotherapy.

Developing a predictive model of responsiveness to 5-FU and oxaliplatin-based chemotherapy in GC. Since existing molecular subtyping methods based on hundreds of genes are less effective, we developed a predictive model of responsiveness to 5-FU and oxaliplatin-based chemotherapy with the fewest possible markers. After developing a predictive model based on 123 DEGs using Bayesian Compound Covariate Predictor (BCCP) algorithm, we reduced to 30 markers with high gene weight to create a simplified model with a correlation coefficient of 0.908 (with cross-validation P value < 0.01, sensitivity for Rs of 0.86 and specificity of 0.89) (Supplementary Figs. 12 and 13). The high-weighted genes of the 30-gene prediction model included genes related to...
Fig. 3 Identification of response-associated gene expression features for 5-FU and oxaliplatin-based chemotherapy. a Gene set enrichment analysis (GSEA) of the R versus NR PDX models, using RNA sequencing data. GSEA demonstrated that metabolism-related pathways were enriched in the R group and Wnt/β-catenin and angiogenesis signaling pathways were enriched in the NR group. Normalized enrichment score (NES) and nominal P value is described. b Identification of core differentially expressed genes (DEGs) between the R and NR groups in both patient tumors and PDX tumors (Top). A total of 40 upregulated genes were identified in the R group and 83 upregulated genes were identified in the NR group. The bottom panel shows the genetic and transcriptomic alterations in TP53-associated genes in the R and NR groups. c Protein–protein interaction enrichment analysis based on STRING network for the 83 DEGs that are upregulated in the NR group. Enrichment P value are corrected for multiple testing using the method of Benjamini and Hochberg.
apoptosis, metabolism, ECM interactions, and EMT (Fig. 5a). The predictive model was applied to other independent GC cohorts to confirm the predictive value of this model by comparing the estimated responsiveness with patients’ outcomes. Patients in cohort of ACRG, TCGA, and Singapore-Duke study did not receive neoadjuvant therapy and some patients received adjuvant therapy with 5-FU-based regimens. Applied to 300 GC patients of ACRG, 165 and 113 patients were assigned to R-like and NR-like, respectively (Supplementary Table 5). Survival analysis showed that NR-like patients had worse prognosis than R-like patients in both relapse-free (P < 0.0001 for all patients and P = 0.061 for patients with adjuvant therapy by log-rank test) and overall survival (P < 0.0001) (Fig. 5b and Supplementary Fig. 14a–c). In 262 TCGA GC patients, patients assigned to R-like (n = 105)
showed significantly better prognosis in both relapse-free and overall survival than patients assigned to NR-like (n = 84) (Fig. 5c, Supplementary Table 5, and Supplementary Fig. 15). In 200 GC patients of Singapore-Duke study, patients were classified to 105 R-like and 79 NR-like subgroups (Supplementary Table 5), and NR-like patients had worse prognosis than R-like patients in overall survival (P = 0.0013 by log-rank test; Fig. 5d and Supplementary Fig. 16). When compared with subtypes in each cohort, most MSI-type samples in the ACRG and TCGA cohorts were classified into R-like groups, and most EMT-type sample in the ACRG cohort and most invasive samples in Singapore-Duke study were assigned to NR-like group (Fig. 5e–g). Taken together, we suggest that 30-gene-based classifier model has a predictive value for FOLFOX responsiveness and GC patient prognosis.

**Discussion**

In this study, we comprehensively investigated genomic and transcriptomic differences between Rs and NRs to 5-FU and oxaliplatin-based chemotherapy, estimated from PDX experiments, and identified several important tumor biology pathways associated with drug response (Fig. 6a, b): (1) defective p53 signaling, in NRs; (2) increased metabolic processes, in Rs; and (3) increased cell-to-cell and cell-to-ECM interactions, in NRs. Defects in the p53 pathway have been suggested as determining factors for the sensitivity of 5-FU-based chemotherapy, in several cancer types, because p53 status is strongly associated with apoptosis and tumor-suppressing effects. In addition, several genes were reported to play roles in the resistance to 5-FU-based chemotherapy by regulating p53-dependent pathway. Our genomic and transcriptomic analyses demonstrated a lower mutation frequency for TP53 and the increased expression of p53 target genes, such as MDM2 and CDKN1A, in the R group (Fig. 3b and Supplementary Fig. 7c). NRs with no TP53 mutation also showed a higher p53 activity (group average: −0.13; Supplementary Fig. 7c), suggesting that p53 function is inactive or defective in most NRs. Furthermore, among 343 genes, directly regulated by p53, several genes including SFRP and FDXR (associated with metabolism; P = 0.0002), and BBX3 (associated with apoptosis; P = 0.0049) were significantly downregulated in the NR group. These data suggest that defective p53 signaling is one of the predictive features for 5-FU and oxaliplatin-based chemotherapy resistance.

Our transcriptomic analyses demonstrated metabolism-related gene set enrichment in the R group and the activation of cell-to-cell and cell-to-ECM interactions in the NR group, which were reproducibly detected in analyses of both cancer cells and the TME (Figs. 3a and 4a), indicating the importance of interactions between cancer and stromal cells in the TME for the development of resistance to 5-FU and oxaliplatin-based chemotherapy. Several soluble factors in the TME appear to play critical roles in determining chemotherapy responsiveness. TGF-β plays an important role in drug resistance against both targeted and conventional agents. Recent studies showed that the treatment of triple-negative breast cancer xenografts with paclitaxel induced autocrine TGF-β signaling, cancer stem cell formation, and drug resistance. Clinical trials are ongoing for the combined treatment of chemotherapeutic drugs and the TGF-β receptor inhibitor LY2157299, in glioblastoma (NCT01582269), hepatocellular carcinoma (NCT01246986), and pancreatic cancer (NCT01373164). Wnt/β-catenin signaling is reportedly involved in a multitude of developmental processes, the regulation of cell proliferation, differentiation, migration, genetic stability, and apoptosis. Although therapeutic agents that specifically target the Wnt pathway have recently entered clinical trials, the detailed mechanisms through which the Wnt pathway generates chemotherapeutic drug resistance remain unclear. TGF-β signaling and Wnt/β-catenin were highly associated with the EMT process. Previous studies have suggested that TGF-β-induced EMT confer GC cells resistance to 5-FU and oxaliplatin, and suppression of EMT via diverse approaches increased the sensitivity to 5-FU-based chemotherapy in GC. The role played by the EMT in reduced therapeutic efficacy is evident in several tumors. The 5-FU-resistant cell lines showed typical EMT alterations, including increased invasiveness, the upregulation of mesenchymal markers, the increased expression of EMT-related proteins, and the downregulation of epithelial markers. Our transcriptomic data showed that several EMT-related genes were significantly upregulated in the NR group (Supplementary Fig. 10a, b), and in our prediction, ACRG EMT subgroup and Singapore-Duke invasive subtypes were mostly assigned to NR-like (Fig. 5e, g). These data emphasized that cancer cells and the TME work cooperatively during resistance to 5-FU and oxaliplatin-based chemotherapy, by activating comparable pathways. The effect of defective MMR pathway in 5-FU-based chemotherapy responsiveness is quite controversial. In previous clinical trials, the CLASSIC and the MAGIC trials demonstrated that patients with MSI high (MSI-H) GC showed no survival benefit and worse survival from 5-FU-based adjuvant chemotherapy, respectively. However, recent study from 162 Korean patients with MSI-H GC demonstrated that the group with 5-FU-based adjuvant chemotherapy showed better overall and disease-free survival compared to the group with no adjuvant chemotherapy, suggesting a possible role of a defective MMR pathway in the responsiveness of 5-FU-based adjuvant chemotherapy. In our data, despite a higher frequency of MSI-H (Rs: 38.5%, NRs: 27.3%) and following higher frequencies of defective MMR mutational signatures in Rs (Fig. 2b, c), those features are not general characteristics of the R group. Recent studies reported molecular subtyping of MSI-H tumors in colorectal and GCs and revealed prognostic difference among MSI-H tumors, suggesting that patients with MSI-H are heterogeneous in terms of tumor characteristics and prognosis. Therefore, the effect of a defective MMR pathway on the responsiveness of 5-FU-based
Fig. 5 Development of a response prediction model for 5-FU-based chemotherapy. a The gene expression of 30 classifier genes from among the core DEGs in PDX tumors. b–d Survival analyses for GC patients in ACRG (b), TCGA (c), and Singapore-Duke (d) studies, according to prediction model. Patients were stratified by our prediction model, and the responder-like (R-like) patient group showed a significantly better prognosis than the non-responder-like (NR-like) group. P values were calculated from the log-rank test. ACRG the Asian Cancer Research Group. e–g Reassignment of GC patients from the ACRG study (e), TCGA (f), and Singapore-Duke (g) studies according to prediction model (Top). Survival analyses for subgroups in ACRG (e), TCGA (f), and Singapore-Duke (g) studies, according to prediction model (bottom). P values were calculated from the two-sided log-rank test.
adjuvant chemotherapy needs to be evaluated by randomized prospective clinical trials with a large cohort of MSI-H GC patients.

The responsiveness prediction model, built using DEGs as biomarkers in a machine learning-based prediction model and 30 gene expression signatures, correctly classified our PDX models and GC patient cohorts. Several efforts have been made to identify GC biomarkers, including a predictive test for adjuvant chemotherapy response, which compared the expression levels of four classifier genes in formalin-fixed, paraffin-embedded tumors between patients who underwent surgery alone and those who received postoperative chemotherapy. However, this study was unable to completely separate the effects of surgery from the effects of chemotherapy. Recently, to identify predictive biomarkers for 5-FU + oxaliplatin-based neoadjuvant chemotherapy, Li et al. compared the sequencing data profiles of biopsy samples collected from 35 Chinese GC patients who underwent neo-adjuvant therapy. By comparing the multi-omics characteristics of 17 Rs and 18 NRs, they demonstrated that MSI and MDM2 amplification were observed in non-responsive tumors, whereas MYC amplification was observed in responsive tumors. Some discrepancies exist between this previous study and our data, especially for the effects of MSI in drug responsiveness. Although our observations were cross-validated in both patient and PDX tumors, detailed explanations regarding these differences must be further evaluated.

PDXs are increasingly used as drug development tools in preclinical settings and have been shown to recapitulate the histology and behavior of the cancers from which they are derived. However, whether PDXs mimic the clinical outcomes of patients remains controversial. The Golub group tracked the dynamics of copy number alterations during passaging in 1110 PDX models from 24 types of cancers, collecting the relevant genetic data. The results revealed variations in the mouse data compared with the genetic data from human tumor cells after the tumor tissues were transplanted into mice. The researchers suggested that these changes may cause different responses to cancer drugs in the PDX models. In contrast, the Sidransky group established PDX models of 92 patients with various solid cancers and conducted a dosing study, which identified correlations as high as 87% between the response to drugs in patients and the associated PDX models. Also, Wong et al. performed a retrospective population

Fig. 6 Summary of integrative characteristics associated with 5-FU-based therapy responding and non-responding tumors. a Representative pathways enriched in the R group. b Representative pathways enriched in the NR group. The figures were generated with BioRender.
pharmacokinetic-pharmacodynamic analysis of relevant xenograft efficacy data for eight anticancer drugs with known clinical outcome, including 5-FU. This group suggested that agents that can achieve 60% TGI or higher in preclinical models are more likely to drive clinical efficacy in man\(^6\). Our data demonstrated that the estimated responsiveness to 5-FU and oxaliplatin-based chemotherapy in PDX models was highly correlated with clinical outcomes in the adjuvant setting (Fig. 1c–e) and that the prediction model, developed from analyses of PDX tumors, was able to predict patients’ drug responsiveness and prognosis (Fig. 5). There are some limitations in analyzing human samples compared to PDX models. It is difficult to obtain large quantity of cancer tissues from patients, and tumor cell purity in tissues are more diverse in human samples than in PDX tumors. Patients’ general conditions and environmental factors are hard to normalize in human study, which requires large size of patient cohort. Therefore, through multiple PDXs, integration of drug responsiveness, and genomic/transcriptomic profiling will provide additional prediction model for treatment responsiveness other than 5-FU and oxaliplatin-based chemotherapy.

Methods

**GC patient sample collection.** This study was approved by the institutional review board of the Seoul National University Hospital (No. C-1402-054-555), in accordance with the Declaration of Helsinki. All samples were obtained with informed consent at the Seoul National University Hospital. Tissue samples of GCs, paired normal gastric tissues, and blood samples were obtained from individuals who underwent gastrectomy at Seoul National University Hospital between 2014 and 2017. Patients who were treated preoperatively with chemotheraphy or chemoradiation therapy were excluded from this study. Immediately after sample acquisition, tumor samples were transferred to RPMI 1640 medium (Thermo Fisher Scientific), containing 1% penicillin/streptomycin (Thermo Fisher Scientific), for PDX generation.

**Generation of PDX models.** Mice were cared for according to the institutional guidelines of the Institutional Animal Care and Use Committee of Seoul National University Hospital (No. 14-0016-CA01). For PDX models, surgically resected tissues were minced into pieces, approximately 2 mm in size, and injected into the subcutaneous area of the flanks of 6-week-old NOD-SCID/IL-2γ-receptor null (NSG mice, The Jackson Laboratory) female mice. The tumor volumes and body weights of the mice were checked once or twice, weekly. Tumor volume was calculated as (length × width\(^2\))/2. The animals were continued to be observed until the tumors reached approximately 200 mm\(^3\). Twenty mutants were included for the analysis. For each tumor, the mutant tissues were divided and stored for three purposes: (1) tumor tissues were cryopreserved in liquid nitrogen for use in the next passage of PDXs; (2) tumor tissues were frozen in liquid nitrogen, for genomic analyses; and (3) tumor tissues were fixed in 10% formalin solution, for histological analyses.

**In vivo pharmacological studies.** In PDX mice bearing subcutaneous engraftments of previously established PDX tumors, drug treatments began after the tumors reached approximately 200 mm\(^3\). Mice were divided randomly, into control and 5-FU + oxaliplatin-treated groups, with five mice in each group. 5-FU (Selleckchem, 5 mg/kg, weekly) and oxaliplatin (Selleckchem, 50 mg/kg, weekly), in saline, was administered via intraperitoneal injection for 21 days. Tumors volumes were checked three times, weekly, calculated as (length × width\(^2\))/2. To evaluate the responsiveness, we combined two criteria comparing relative tumor volume changes between drug- and vehicle-treated arms: (1) drug treatment significantly inhibited tumor growth [two-way ANOVA P < 0.0001 for the R group, P > 0.05 for the NR group]; and (2) the TGI (%) was >60% for the R group (Supplementary Table 2). TGI is calculated via the following equation:

\[
\text{TGI} (%) = \frac{TV_{\text{vehicle}} - TV_{\text{treatment}}}{TV_{\text{vehicle}} - TV_{\text{initial}}} \times 100
\]  

where \(TV_{\text{vehicle}}\) is the tumor volume for the vehicle-treated animals at a specified end point time, \(TV_{\text{initial}}\) is the initial tumor volume at the start of the treatment, and \(TV_{\text{treatment}}\) is the tumor volume of the drug treatment groups at a specified end point time. The intermediate group consist of samples that did not meet these criteria. The ANOVA test was performed using the SPSS software version 22 (IBM Corp., Version 22.0).

**Statistical analysis of clinical characteristics.** Clinical and pathological information regarding enrolled patients was collected prospectively. The following variables were analyzed to identify factors associated with responsiveness to 5-FU and oxaliplatin-based chemotherapy: (1) the patient’s age and sex, and (2) tumor pathologic characteristics, including pTNm stage, according to the Seventh Edition of the Union for International Cancer Control, Lauren classification. Continuous variables are presented as the median and analyzed by Mann–Whitney U test, using the SPSS software. Categorical variables are presented as the number and percentage and analyzed by Fisher’s exact test. A P value of <0.05 was considered significant.

**Isolation of nucleic acids and next-generation sequencing.** Genomic DNA was extracted from patient blood samples using the Gentra Puregene Blood Kit (Qiagen), and tumor DNA was obtained from patients and PDX tumors using the DNeasy Blood & Tissue Kit (Qiagen). Then 250 ng of DNA was sonicated with a Covaris S2200 Focused-ultrasonicator, and 101-bp paired-end libraries were constructed with the SureSelect All Exon V5 Kit (Agilent). WES was performed on Illumina HiSeq 2000 instruments, with read lengths of 2 × 101 bp.

RNA extraction from non-tumor tissues and patient and PDX tumors was performed using Trizol® (Invitrogen). Samples with an RNA integrity number >5 were further processed. The 101-bp paired-end libraries were constructed with the TrueSeq RNA Sample Prep Kit v2 (Illumina), using 1 µg of RNA. Whole-transcriptome sequencing (WTS) was performed on Illumina HiSeq 2000 instruments.

**Sequencing data processing using combined reference.** We built a combined reference genome of the human (GRCh37) and mouse reference genomes (GRCm38) and raw FASTQ files from WES and WTS were aligned with the combined reference genome. Sequenced reads from WES and WTS were aligned using a Burrows-Wheeler Aligner mem\(^6\) and STAR aligner\(^6\), respectively. The sorting and marking of duplicates were performed by Picard tools. After processing the binary aligned and mapping (BAM) files, mouse genome-aligned reads were removed for analyses of human cancer cells.

**Identification of somatic single-nucleotide variants and insertions/deletions (indels).** Indel realignment and base recalibration of the BAM files were performed using the Genome Analysis Tool Kit (GATK) version 3.2. Somatic mutations were called using MuTect\(^6\) and Indelocator\(^2\) and annotated with Annovar\(^8\). Variants with total read depths <7 and alternate allele depth <4 were excluded. To select rare functional mutations, coding sequence mutations, with a population frequency of <0.01, were included, based on the ESP6500 and the total and East Asian populations of 1000 Genomes Project phase 3 and ExAC studies. SNPs with ≥1% minor allele frequency, based on the dbSNP flag, and error-prone variants on the segmentally duplicated region were removed.

Cancer-related genes were annotated using cancer gene databases, including TARGET (Tumor Alterations Relevant for GEnomics-driven Therapy; http://www.broadinstitute.org/cancer/cga/target), Cancer Gene Census\(^1\), and known cancer genes from Vogelstein et al\(^8\). Oncogenes and oncogenic/likely oncogenic mutations were annotated by OncoKB (http://www.oncokb.org).

**Decomposition of mutational signatures.** We estimated the amount of exposure to mutational processes for each tumor sample using MuTect results, based on the mutational signatures of the COSMIC database (http://cancer.sanger.ac.uk/cosmic/signatures). The R package deconstructSigs\(^6\) was used to compute the mutational signatures, and tumor samples with matched germline data that harbored at least 20 mutations were included for the analysis.

**Analysis of clonal architecture.** To estimate tumor heterogeneity and changes in the clonal architecture of each pair of patient and PDX tumors, PyClone, a Bayesian clustering method relying on Markov Chain Monte Carlo, was applied\(^8\). Based on the allele frequencies of somatic mutations and copy number values from WES, PyClone clustered mutations that shifted together across tumors and predicted the cellular prevalence of each cluster. A beta-binomial emission was applied. Two pairs of patient and PDX tumors were excluded from this analysis due to the absence of a matched patient tumor and few mutational identities between the patient and PDX tumors.

**Identification of SCNAs.** To identify SCNAs between Rs and NRs that may affect drug response, 2-transformed RPKMs (Reads Per Kilobase per Million mapped reads) of each tumor were calculated using CoNIFER\(^2\), log normalized with matched germline data, and further segmented using DNA copy package (https://www.biocorductor.org/packages/release/bioc/html/DNAcopy.html). For tumor samples without matched blood, pooled blood data were used as a baseline. Amplification was defined as a value >0.2, whereas deletion was defined as a value < −0.2.

**Gene expression analysis.** The numbers of expressed reads for each gene were quantified using the HTSeq-count program, based on the GRCh37 Ensemble v65. Fragments per kilobase of exon per million mapped reads (FPKM) values were calculated and normalized with edgeR\(^8\). Of the 62,000 Ensemble genes, we first...
removed genes with median FPKM values < 1. GSEA between Rs and NRs was performed on GenePattern module, using the Hallmark gene sets. The extraction of DEGs was performed using DESeq2 package, which uses raw counts. After DEG extraction, we applied further filtration steps as follows: adjusted P value < 0.1, log fold-change ≥ 1.5. Using filtered DEGs, we performed pathway analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis tool and Gene Ontology database and determined statistical significance with The Database for Annotation, Visualization and Integrated Discovery functional annotation tool. Clustering was performed based on log-transformed and gene-centered FPKM values, using the cluster3.0 program, and visualized with Treeview. Enrichment of PPIs among DEGs was analyzed based on KEGG, using the STRING database.

TME analysis. During the xenotransplantation, human stromal cells are replaced with mouse-derived stromal cells, and PDX tumors composed of human tumor cells become surrounded by murine stroma. Thus, tumor-specific gene expression was observed through genome-aligned reads, and stroma-specific gene expression could be separately analyzed based on mouse genome-aligned reads.

Functional enrichment of PPIs among DEGs was performed using the STRING database. Enrichment of PPIs among DEGs was analyzed based on KEGG, using gene-centered FPKM values, using the cluster3.0 program, and visualized with Treeview. Enrichment of PPIs among DEGs was analyzed based on KEGG, using the STRING database.

Development of the response prediction model to 5-FU and oxaliplatin-based chemotherapy. To develop a predictive classification model for 5-FU-based chemotherapy, we modeled the expression of 64 stromal and immune cell types. We converted mouse genes to orthologous human genes, and xCell was used to determine the microenvironment components of PDX tumors compared with those in patient tumors.

Data availability
The PDX sequencing data generated in this study have been deposited in the European Nucleotide Archive (ENA) repository under accession code: PRJEB40936. The TCGA validation data used in this study are available in the FIREHOUSE database [http://firebrowse.org/?c=thornt=STAD]. The ACRG expression data used in this study are available in the Gene Expression Omnibus (GEO) database under accession code: GSE62254. The Singapore-Duke gene expression data used in this study are available in the GEO database under accession code: GSE15495. Source data are provided with this paper.

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References
1. Bray, F. et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Ca. Cancer J. Clin. 68, 394–424 (2018).
2. Cristescu, R. et al. Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. Nat. Med. 21, 449–456 (2015).
3. Ajani, J. A. Evolving chemotherapy for advanced gastric cancer. Oncologist 10 (Suppl 3), 49–58 (2005).
4. Hermann, R. M., Rave-Frank, M. & Pradier, O. Combining radiation with oxaliplatin: a review of experimental results. Cancer Radiother. 12, 61–67 (2008).
5. Kim, H. K. et al. A gene expression signature of acquired chemoresistance to cisplatin and fluorouracil combination chemotherapy in gastric cancer patients. PLoS ONE 6, e16694 (2011).
6. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. Nature 513, 202–209 (2014).
7. Lei, Z. et al. Identification of molecular subtypes of gastric cancer with different responses to PI3-kinase inhibitors and 5-fluorouracil. Gastroenterology 145, 1554–1565 (2013).
8. Roth, A. et al. PyClone: statistical inference of clonal population structure in cancer. Nat. Methods 11, 396–398 (2014).
9. Wong, H. et al. Antitumor activity of targeted and cytotoxic agents in murine subcutaneous tumor models correlates with clinical response. Clin. Cancer Res. 18, 3846–3855 (2012).
10. Zhao, T. et al. The effects of genomic polymorphisms in one-carbon metabolism pathways on survival of gastric cancer patients received fluorouracil-based adjuvant therapy. Sci. Rep. 6, 28019 (2016).
11. Des Guetz, G. et al. Microsatellite instability and sensitivity to FOLFOX treatment in metastatic colorectal cancer. Anticancer Res. 27, 2715–2719 (2007).
12. Azan, A. et al. Impact of 5p3 expression and microsatellite instability on stage III colon cancer disease-free survival in patients treated by 5-fluorouracil and leucovorin with or without oxaliplatin. Ann. Oncol. 21, 772–780 (2010).
13. Jo, W. S. & Carethers, J. M. Chemotherapeutic implications in microsatellite unstable colorectal cancer. Cancer Biomark. 2, 51–60 (2006).
14. Lowe, S. W., Bleyer, H. E., Jacks, T. & Housman, D. E. p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell 74, 957–967 (1993).
15. Osaki, M. et al. 5-fluorouracil (5-FU) induced apoptosis in gastric cancer cell lines: role of the p53 gene. Apoptosis 2, 221–226 (1997).
16. Alexandrov, L. B. et al. Signatures of mutational processes in human cancer. Nature 500, 415–421 (2013).
17. Barak, Y., Juven, T., Haffner, R. & Oren, M. Mdm2 expression is induced by wild-type p53 activity. EMBO J. 12, 461–468 (1993).
18. Bouvard, V. et al. Tissue and cell-specific expression of the p53-target genes: bax, fas, mdm2 and waf1/p21, before and following ionising irradiation in mouse mammary tumor. Int. J. Cancer 61, 542–551 (1995).
19. Loboda, A. et al. EMT is the dominant trait in human colon cancer. BMC Med. Genomics 4, 9 (2011).
20. Oh, S. C. et al. Clinical and genomic landscape of gastric cancer with a mesenchymal phenotype. Nat. Commun. 9, 1777 (2018).
21. Chao, C. et al. Patient-derived xenografts from colorectal cancer: a temporal and hierarchical study of murine stromal cell replacement. Anticancer Res. 37, 3405–3412 (2017).
22. Cho, S. Y. et al. Unstable genome and transcriptome dynamics during tumor metastasis contribute to therapeutic heterogeneity in colorectal cancers. Clin. Cancer Res. 25, 2821–2834 (2019).
23. Bradford, J. R. et al. Whole transcriptome profiling of patient-derived xenograft models as a tool to identify both tumor and stromal specific biomarkers. Oncotarget 7, 20773–20787 (2016).
24. Ge, A. Z. & Butcher, E. C. Cloning and expression of a cDNA encoding mouse endoglin, an endothelial cell TGF-beta ligand. Gene 138, 201–206 (1994).
25. Elyaman, W. et al. Notch receptors and smad3 signaling cooperate in the induction of interleukin-9-producing T cells. Immunity 36, 623–634 (2012).
26. Pietras, A., von Stedingk, K., Lindgren, D., Pahlman, S. & Axelson, H. JAG2 induction in hypoxic tumor cells alters notch signaling and enhances endothelial cell tube formation. Mol. Cancer Res. 9, 626–636 (2011).
27. Tian, H. Y. et al. Endoglin interacts with VEGFR2 to promote angiogenesis. Cancer Res. 72, 626–34 (2012).
28. Bhola, N. E. et al. TGF-beta inhibition enhances chemotherapy action against murine breast cancer via TGF/Smad2-induced epithelial-mesenchymal transition. Mol. Cancer Res. 13, 10–21 (2015).
29. Han, Y. et al. HOXA13 contributes to gastric carcinogenesis through DHRS2 and leucovorin with or without oxaliplatin. Anticancer Res. 23, 461–468 (2007).
30. Chakravarti, A. et al. Comprehensive molecular characterization of gastric adenocarcinoma. Nature 513, 202–209 (2014).
31. Wada, T. et al. HIF-1alpha enhances in vitro motility of glioblastoma cells by activation of ZEB1 and TGF-beta. J. Neurooncol. 123, 1348–1358 (2013).
32. Zavadil, J. & Bottinger, E. P. TGF-beta and epithelial-to-mesenchymal transition. Cytokine Growth Factor Rev. 24, 576–587 (2013).
33. Kallweit, U. D. et al. Activation of canonical Wnt/beta-catenin signaling enhances in vitro motility of glioblastoma cells by activation of ZEB1 and other activators of epithelial-to-mesenchymal transition. Cancer Lett. 325, 42–53 (2012).
34. Zheng, H. Z. et al. Hylauran-mediated motility receptor confers resistance to chemotherapy via TGF/Smad2-induced epithelial-mesenchymal transition in gastric cancer. Cancer Res. 73, 6365–6377 (2019).

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37. Li, L. Q. et al. Sensitization of gastric cancer cells to 5-FU by microRNA-204 through targeting the TGFBR2-mediated epithelial to mesenchymal transition. *Cell Physiol. Biochem.* 47, 1533–1545 (2018).
38. Kurata, T. et al. Low-dose eribulin mesylate exerts antitumor effects in gastric cancer by inhibiting fibrosis via the suppression of epithelial-mesenchymal transition and acts synergistically with 5-fluorouracil. *Cancer Manag. Res.* 10, 2729–2742 (2018).
39. Wu, D. M. et al. MicroRNA-17 inhibition overcomes chemoresistance and suppresses epithelial-mesenchymal transition through a DEDD-dependent mechanism in gastric cancer. *Int. J. Biochem. Cell Biol.* 102, 59–70 (2018).
40. Jiborn, T., Bjartell, A. & Abrahamsen, P. A. Neuroendocrine differentiation in prostatic carcinoma during hormonal treatment. *Urology* 51, 585–589 (1998).
41. Arumugam, T. et al. Epithelial to mesenchymal transition contributes to drug resistance in prostate cancer. *Cancer Res.* 69, 5820–5828 (2009).
42. Wang, Z. W. et al. Acquisition of epithelial-mesenchymal transition phenotype of gemcitabine-resistant pancreatic cancer cells is linked with activation of the notch signaling pathway. *Cancer Res.* 69, 2400–2407 (2009).
43. Lund, K. et al. Slug-dependent upregulation of LIMCAM is responsible for the increased invasion potential of pancreatic cancer cells following long-term 5-FU treatment. *PLoS ONE* 10, e0125648 (2015).
44. Kim, S. T. et al. Clinical impact of microsatellite instability in colon cancer following adjuvant FOLFOX therapy. *Cancer Chemother. Pharmacol.* 66, 659–667 (2010).
45. Smyth, E. C. et al. Mismatch repair deficiency, microsatellite instability, and survival: an exploratory analysis of the National Cancer Research Institute Adjuvant Gastric Infusion Chemotherapy (MAGIC) trial. *JAMA Oncol.* 3, 1197–1203 (2017).
46. Choi, Y. Y. et al. Microsatellite instability and programmed cell death-ligand 1 expression in stage II/III gastric cancer: post hoc analysis of the CLASSIC randomized controlled study. *Ann. Surg.* 270, 309–316 (2019).
47. Kim, J. W. et al. Adjuvant chemotherapy in microsatellite instability-high gastric cancer. *Cancer Res. Treat.* 52, 1178–1187 (2020).
48. Hu, W. et al. Subtyping of microsatellite instability-high colorectal cancer. *Cell Commun. Signal.* 17, 79 (2019).
49. Yang, Y., Shi, Z., Bai, R. & Hu, W. Heterogeneity of MSI-H gastric cancer identifies a subtype with worse survival. *J. Med. Genet.* 58, 12–19 (2021).
50. Cheong, J. H. et al. Predictive test for chemotherapy response in resectable gastric cancer: a multi-cohort, retrospective analysis. *Lancet Oncol.* 19, 629–638 (2018).
51. Li, Z. Y. et al. Multi-omics characterization of molecular features of gastric cancer correlated with response to neoadjuvant chemotherapy. *Sci. Adv.* 6, eaay4211 (2020).
52. Ben-David, U. et al. Patient-derived xenografts undergo mouse-specific tumor evolution. *Nat. Genet.* 49, 1567–1575 (2017).
53. Izumchenko, E. et al. Patient-derived xenografts effectively capture responses to oncology therapy in a heterogeneous cohort of patients with solid tumors. *Ann. Oncol.* 28, 2595–2605 (2017).
54. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25, 1754–1760 (2009).
55. Dobin, A. et al. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 29, 15–21 (2013).
56. Cibulskis, K. et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nat. Biotechnol.* 31, 213–219 (2013).
57. Banerji, S. et al. Sequence analysis of mutations and translocations across breast cancer subtypes. *Nature* 486, 405–409 (2012).
58. Wang, K., Li, M. & Hakonarson, H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res.* 38, e164 (2010).
59. Sondka, Z. et al. The COSMIC Cancer Gene Census: describing genetic dysfunction across all human cancers. *Nat. Rev. Cancer* 18, 696–705 (2018).
60. Vogelstein, B. et al. Cancer genome landscapes. *Science* 339, 1546–1558 (2013).
61. Rosenthal, R., McGranahan, N., Herrero, J., Taylor, B. S. & Swanton, C. DeconstructSeq: delineating mutational processes in single tumors distinguishes DNA repair deficiencies and patterns of carcinoma evolution. *Genome Biol.* 17, 31 (2016).
62. Krumm, N. et al. Copy number variation detection and genotyping from exome sequence data. *Genome Res.* 22, 1525–1532 (2012).
63. Robinson, M. D., McCarthy, D. J. & Smyth, G. K. edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26, 139–140 (2010).
64. Reich, M. et al. GenePattern 2.0. *Nat. Genet.* 38, 500–501 (2006).
65. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15, 550 (2014).
66. Callari, M. et al. Computational approach to discriminate human and mouse sequences in patient-derived tumour xenografts. *BMJ Genomics* 19, 19 (2018).
67. Aran, D., Hu, Z. & Butte, A. J. xCell: digitally portraying the tissue cellular heterogeneity landscape. *Genome Biol.* 18, 220 (2017).
68. Simon, R. et al. Analysis of gene expression data using BRB-ArrayTools. *Cancer Inform.* 3, 11 (2007).

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Author contributions
S.-A.I., H.-K.Y., C.L. and J.-I.K. conceived the study. D.N., J. Chae, S.-Y.C., A.M., S.-A.I., H.-K.Y., C.L. and J.-I.K. designed the study. A.M., Y.-J.K., K.-H.L., T.-Y.K., Y.-S.S., S.-H. K., H.-J.L., S.-A.I., and H.-K.Y. collected patient tumor samples. D.N., J. Chae, S.-Y.C., W.K., A.L., S.M., I.K. M.J.K., J. Choi, W.L., D.S., and A.M. performed analysis. D.N., J. Chae, S.-Y.C., A.M., K.-H.L., T.-Y.K., Y.-S.S., S.-H. K., H.-J.L., S.-A.I., and H.-K.Y. collected patient tumor samples. D.N., J. Chae, S.-Y.C., A.M., K.-H.L., T.-Y.K., Y.-S.S., S.-H. K., H.-J.L., W.-H.K., H.P., S.-A.I., H.-K.Y., C.L. and J.-I.K. interpreted results. D.N., J. Chae, and S.-Y.C. wrote initial draft. D.N., J. Chae, S.-Y.C., A.M., S.-A.I., H.-K.Y., C.L., and J.-I.K. contributed to writing and editing the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
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