Methods

Human tissue samples

De-identified human gastric cancer tissues and adjacent non-cancer tissues from 36 patients were collected at Fujian Medical University Union Hospital (FJMUUH, Fuzhou, China) for immunohistochemistry and whole-transcriptome analysis (Supplementary Table 1). The RNA-sequencing was performed by Kangchen Biotechnology Co., Ltd. (Shanghai, China) using TruSeq SR Cluster Kit v3-cBot-Hs (#GD-401-3001, Illumina) in Illumina HiSeq4000. Frozen gastric cancer samples (14 cases) for Western blots were collected in Vanderbilt University and University of Miami (Supplementary Table 6). The institutional review board approved the use of de-identified human specimens in the study. The informed consent was obtained from the subjects. The study complied with the principles outlined in the Declaration of Helsinki.

Public datasets collection and collation

To investigate the clinical molecular characteristics and prognosis among immunophenotypes, gene expression RNA-sequencing data for The Cancer Genome Atlas (TCGA) datasets, including stomach (STAD), breast (BRCA), skin (SKCM), lung (LUAC), ovary (OV), colon (COAD), and esophagus (EAC), were downloaded from UCSC Xena browser (GDC hub://gdc.xenahubs.net). The gene expression data and the corresponding clinical-pathological data of GSE66229 (ACRG cohort) were obtained from the Gene Expression Omnibus (GEO, https: //www.ncbi.nlm.nih.gov/gds/). To evaluate the responses to immunotherapy among various immunophenotypes, we utilized the RNA sequencing data of gastric cancer (ENA cohort) from the European Nucleotide Archive (ENA, PRJEB25780), the RNA sequencing data of urinary tract tumors (IMvigor210 cohort) from http://research-pub.gene.com/IMvigor210CoreBiologies, and three datasets with various cancer types (GSE165252, GSE91061, and GSE35640) from GEO. RNA-sequencing data were transformed from Fragments per Kilobase of transcript per Million (FPKM) values into Transcripts Per kilobase Million (TPM) values for more comparable among different samples and various datasets. The Cancer Proteome Atlas (TCPA) was employed to investigate the protein expression. Data were analyzed with the R software (version 4.1.0) and R packages.

Functional pathway enrichment analysis

Gene set enrichment analysis (GSEA) was performed using the "clusterProfiler" R package[1] to calculate the normalization enrichment score (NES) and the false discovery rate (FDR) of each signature gene set. The differential expressed genes were identified by the "limma" R package. The reference signature gene sets, including hallmark gene sets and GO biological processes gene sets, were downloaded from the MSigDB database of the Broad Institute (https://www.gsea-msigdb.org/). Each gene set was considered significant when the FDR was less than 25%.

Unsupervised clustering

Bulk samples with qualitatively differential signature gene sets were classified using hierarchical clustering based on Euclidean distance. Unsupervised clustering methods (K-means) were performed to identify predicted immunophenotypes (predicted IP) in all cohorts. To assess the stability of the predicted IP, a consensus clustering algorithm was employed to explore the optimal number of clustering. All clustering algorithms were performed by the "cluster" and "NbClust" R package.

Predicted immunophenotype (predicted IP)

To predict immunophenotypes of tumor samples from public databases, we established an algorithm based on the T cell infiltration signature[2] (Supplementary Table 2) and the EMT signature[3]. Single-Sample Gene Set Enrichment Analysis (ssGSEA) [4] were performed to quantify the enrichment score for the 18 genes-based T cell signature using "GSVA" R package. The EMT signature gene sets, including 315 genes for tumor samples and 218 genes for cell lines (Supplementary Table 2), as well as their weights, were used to calculate EMT scores. For each sample, the correlation between mRNA values and weights of EMT gene sets was measured by using "cor" R package (method= "spearman," use= "complete.obs"). Finally, the formula to calculate the relative EMT score is as following: EMT score = {r-min(r)}/max(r), where r is the correlation coefficient.

Receiver operating curve analysis

A random forest (RF) classifier was performed to discriminate the samples in the training set and validated sets using the "randomForest" R package, and the number of ntree and mtry in RF were set to a default value. The variables for RF included T cell signature and EMT signature. Subsequently, to

evaluate the discrimination performance, the "pROC" R package was employed to calculate the area under the curve (AUC) using receiver operating curve (ROC) analysis. The procedure was repeated 1,000 times to ensure the stability of classification.

Cell culture and reagents

Human gastric cancer cell line HGC-27 was purchased from AMS Biotechnology (AMSBIO, AMS.EP-CL-0107, Switzerland). Human gastric cancer cell lines Hs 746T, AGS and SNU-1 were obtained from American Tissue Culture Collection (ATCC, Manassas, VA). The cell lines MKN28 and MKN45 were purchased from the Riken Cell Bank (Tsukuba, Japan). The human gastric cancer cell line STKM2 and the human gastric epithelium cell line H-GEC were a generous gift from Dr. Alexander Zaika, University of Miami. The mouse gastric cancer cell line YTN2 and YTN16 were a generous gift from Dr. Sachiyo Nomura, University of Tokyo[5, 6]. AGS was cultured in Ham's F-12 Nutrient Mixture (GIBCO, Carlsbad, CA) containing 1% penicillin/streptomycin (GIBCO) and 10% fetal bovine serum (Invitrogen Life Technologies, Carlsbad, CA). Hs 746T was maintained in DMEM medium with 10% fetal bovine serum and 1% penicillin/streptomycin. HGC-27, SNU-1, MKN28, MKN45 and STKM2 were maintained in RPMI/1640 medium with 10% fetal bovine serum and 1% penicillin/streptomycin. YTN2 and YTN16 were maintained in Dulbecco's modified Eagle's medium (DMEM, D6429, Sigma-Aldrich) with 10% fetal bovine serum, 1% penicillin/streptomycin and MITO+ serum extender (No. 355006, Thomas Scientific). All these cell lines were cultured at 37 °C in a humidified incubator with 5% CO2. Mycoplasma infection was routinely examined once a month. All reagents and antibodies used in the study were listed in Supplementary Table 3 and 4.

Immune cell abundance valuation

To evaluate the immune cell abundance from bulk samples, the Immune Cell Abundance Identifier (ImmuCellAI[7], http://bioinfo.life.hust.edu.cn/ImmuCellAI#!/) and CIBERSORT (https://cibersort.stanford.edu/) were employed to evaluate the relative immune cell infiltration abundance for each sample from gastric cancer cohorts based on gene expression data, respectively.

Immunofluorescence and immunohistochemistry staining

Immunofluorescence and immunohistochemistry were performed as previously described[8]. Detailed

antibodies information is included in the Supplementary Table 4.

Transfection and lentivirus infection

Plasmids or siRNA transfection and lentivirus packing/titration were performed as previously described[8]. Detailed information about plasmids, siRNA, and reagents is included in the Supplementary Table 3.

CRISPR/Cas9-Mediated Knockdown of Ifngr1

Ifngr1 was knockout from YTN16 cells via transient transfection using PL-CRISPR.EFS.tRFP-gIfngr1-2 plasmid[9]. The guide sequences 5'-ACTTGAACCCTGTCGTATGC-3' and 5'-TGGTATTCCCAGCATACGAC-3' for mouse IFNGR1. Following transfection and transient selection with puromycin for 3 days, the cells were seeded into 96-well plates for RFP. Knockout clones were selected by Western blots.

CellTiter-Glo luminescence assay

CellTiter-Glo luminescence assay (Promega, Madison, WI) was performed to determine IC50 and drug dose-response curves for each cell line followed drug treatment as previously described[8, 10]. Detailed drugs and reagents information was shown in the Supplementary Table 3.

Wound healing assay

The wound healing assay were performed in 6-well plates using HGC-27 cells followed dovitinib treatment (1.0μM) for 24h. After scratching with a 10-μl pipette, the wound distance was photographed at 0, 12, 18, and 24h and the percentage of wound distance at each time point was calculated compared to that in 0h.

Invasion and migration assays

The invasion and migration assays were determined as previously described[11]. HGC-27 cells were pretreated with dovitinib (1.0μM) or DMSO for 24h. Detailed antibodies and kits information was listed in the Supplementary Table 3 and 4.

Enzyme-linked immunosorbent assay (ELISA)

The protein level of CXCL9 or IFN-γ in the media from both control group and the dovitinib treatment group were examined by the enzyme immunoassay kits obtained from R&D Systems (Minneapolis, MN, USA) according to the manufacturer's instructions. The results were expressed as pg per ml medium. Detailed kits information was listed in the Supplementary Table 3.

Chromatin immunoprecipitation (ChIP)

ChIP assay was determined as previously described[8]. Briefly, ChIP assay was performed with lysates from HGC-27 or AGS with or without overexpression of SNAI1/2 using ChIP-IT Express Enzymatic and ChIP-IT High Sensitivity kit (Active Motif Inc, Carlsbad, CA) according to the manufacturer's instructions. SYBR green qRT-PCR was employed using human IFNG primers (IFNG-P1 and IFNG-P2, Supplementary Table 5).

Western blots and quantitative PCR

Western blots and quantitative PCR (qPCR) analysis was performed using laboratory standard methods as previously described[12]. Detailed antibodies and primers information was listed in the Supplementary Table 4 and 5.

Dual luciferase reporter assay

Cells were seeded in 24-well plates overnight and then transfected with the indicated plasmids using Polyjet reagents following the manufacturer's instruction (Signagen Laboratories, Frederick, MD). Briefly, 1μg of each experimental plasmid (pLX317-SNAI1, SlugMyc_pcDNA3, IFN-gamma luciferase plasmid) and 500 ng of pSV-β-galactosidase plasmid were transected in each transfection. Luciferase activity and β-galactosidase activity were measured after 48 hours of incubation. The results were expressed as relative luciferase activity: firefly luciferase activity divided by β-galactosidase activity.

Peripheral blood lymphocytes isolation and activation

Peripheral blood mononuclear cells (PBMC) were isolated from healthy donors by elutriation with Ficoll-Paque® PLUS Medium (95021-205, VWR). CD3+ T cells were further separated from PBMC

by incubation with biotinylated CD3 antibody (Cat. 13-0037-82, Thermofisher scientific) and Streptavidin MicroBeads (Cat. 130-048-102, Miltenyibiotec) and collection from column on magnet. To activate T cells, the isolated T cells were suspended in complete DMEM medium by supplementing recombinant IL-2 protein (100 ng/ml) and anti-CD28 antibody (5 ug/ml) and cultured in the 6-well plates precoated with anti-CD3 antibody (2 ug/ml) for 72h.

Chemotaxis assay

Cancer cells were seeded in the 6-well plates overnight and then treated with dovitinib (1.0 μM) or recombinant IFN-γ protein (100 ng/ml) or blocking with anti-IFN-γ antibody/normal IgG. After 24h treatment, the supernatants were collected and placed in the bottom of a Transwell migration chamber. The isolated T cells (1×106/ml) from PBMC were added in the top chamber (with 3.0 μm pore). Migration was evaluated after 24h by quantification of the migrated T cells number in the bottom chamber using cell counter (TC20TM, Bio-Rad).

T cell-mediated tumor cell killing assays

During the T cell activation, cancer cells were allowed to adhere to 96 well plates (500 cells per well), and then incubated with activated T cells in the presence or absence of dovitinib (1.0 μM) for 48h. The proportion between activated T cells and cancer cells was 10:1. After removing T cells and cell debris with PBS buffer, cancer cells were re-incubated by adding fresh DMEM medium overnight. The cell viability was measured using the CellTiter-Glo Luminescent Cell Viability kit (Promega, Madison, WI) at luminescence microplate reader (BMG Labtech) according to the manufacturer's instructions.

Syngeneic tumor model

Mouse gastric cancer cells (YTN2 and YTN16) [5, 6] were suspended in the mixture of 50% PBS and 50% Matrigel. C57BL/6 mice were inoculated subcutaneously with the cancer cells (5×106/site) into the flank regions. Mice were randomized into a control group (CTRL) or a treatment group when the average tumor volume reached around 150 mm3. Dovitinib (40mg/kg) was administered daily by oral gavage for 3 weeks. In the combination group, the mice were intraperitoneally (I.P.) injected with 10mg/kg cytotoxic T-lymphocyte-associated protein 4 monoclonal neutralizing antibody (CLTA-4

mAb, BE0032, BioXcell) or the IgG isotype control (BE0091, BioXcell) twice per week for 3 weeks. Tumor growth was monitored twice per week with calipers, and tumor volume was calculated by the formula L×W2×0.5, where L and W represent length and width, respectively. Survival events were scored when tumor volume reached 1000 mm3 or more than 10% body weight lost.

Flow cytometry analysis

The mouse tumor and spleen tissue samples were immediately collected after euthanasia. The single-cell suspension of mouse tissues was obtained by digestion of collagenase A (C9891, Sigma-Aldrich), Trypsin-EDTA (T3924, Sigma-Aldrich), and 40 μm cell strainer filtration. The cells were stained with indicated antibodies of cell surface markers in Flow Cytometry Staining Buffer (Cat. 00-4222, Thermo Fisher Scientific) for 20 min on ice. Following fixation and permeabilization by Intracellular Fixation & Permeabilization Buffer Set (Cat. 88-8824, Thermo Fisher Scientific), intracellular GZMB was stained for 20 min at room temperature. The stained cells were analyzed by an LSR-Fortessa-HTS analyzer (BD Science, San Jose, CA, USA). Data were further analyzed by FCS Express 7.0 software. All flow cytometry antibodies and agents' information were listed in Supplementary Tables 3 and 4.

Statistical analysis and survival analysis

Data are presented as means ± standard deviation (SD) of three independent experiments. Statistical significance of difference between control groups and treatment groups was determined using One-way ANOVA Newman-Keuls test among three groups or more and using Student's t-test between two groups. Pearson's correlation was conducted to test the correlation between two continuous variables. For survival analysis, the Kaplan-Meier method and the log-rank test were employed to evaluate overall survival (OS), recurrence-free survival (RFS) and disease specific survival (DSS). The differences were considered statistically significant when the $p \leq 0.05$.

Data availability

Data are available in public, open access repositories. ENA GC cohort RNAseq data are available in European Nucleotide Archive (https://www.ebi.ac.uk/ena/browser/home); the data of RNA expression profiles and clinical information of GC and pan-cancer available in The Cancer Genome

Atlas (TCGA) official website (https://portal.gdc.cancer.gov/repository); the Gene Expression Omnibus (GEO) datasets are available from National Center for Biotechnology Information (NCBI) GEO database (https://www.ncbi.nlm.nih.gov/). Source data are provided with this paper. All other data are available on reasonable request.

Code availability

All the code will be available from the corresponding authors on reasonable request, including but not limited to the following: RNAseq analysis, GSEA, predicted immunophenotype (predicted IP) algorithm, and unsupervised clustering.

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