Supplementary Methods

Isolation of single cells from GC tissues

Fresh tissues were washed 3 times with Hank's solution containing 1% fetal calf serum before being cut into small pieces. The specimens were then collected in RPMI-1640 medium containing 1 mg/ml collagenase Ⅳ and 10 mg/ml deoxyribonuclease Ⅰ and mechanically dissociated using the gentle MACS Dissociator (Miltenyi Biotec). Dissociated cell suspensions were further incubated for 1 h at 37°C under continuous rotation. The cell suspensions were then filtered through a 70 μm cell strainer (BD Labware). Cell viability, as determined by trypan blue exclusion staining, was typically >90%.

Immunohistochemistry

Paraformaldehyde-fixed and paraffin-embedded samples were cut into 5 μm sections. For immunohistochemical single-staining, the sections were incubated with mouse anti-human CD15, anti-human proliferating cell nuclear antigen (PCNA), or rabbit anti-human CD3 antibodies respectively, and then were stained by horseradish peroxidase (HRP) anti-mouse immunoglobulin G (IgG) or using EnVision G2 System/AP Rabbit/Mouse (Permanent Red) followed by diaminobenzidine. All the sections were finally counterstained with haematoxylin and examined using a microscope (Nikon Eclipse 80i; Nikon).

Immunofluorescence

Paraformaldehyde-fixed sections of tumour tissues from GC patients were washed in PBS and blocked for 30 min with 20% goat serum in PBS and stained for CD15, and CD3 and/or EpCam, and GM-CSF. Slides were examined with a confocal fluorescence microscope (LSM 510 META, Zeiss).

Flow cytometry

Flow cytometric analysis was performed according to standard protocols. For intracellular cytokine measurements, the cells were stimulated for 5 h with phorbol myristate acetate (50 ng/ml) plus ionomycin (1 μg/ml) in the presence of GolgiStop. Intracellular cytokine staining was performed

after fixation and permeabilization using Perm/Wash solution. The cells were analyzed by multicolour flow cytometry with FACSCanto II (BD Biosciences). Data were analyzed with Flowjo software (TreeStar) or FACSDiva software (BD Biosciences).

Real-time PCR

RNA of mouse tumours was extracted using PureLink™ FFPE Total RNA Isolation Kit (Invitrogen). The RNA samples were reversed transcribed to cDNA with PrimeScriptTM RT reagent Kit (TaKaRa). Primers and probes for granzyme B and perforin were obtained from ThermoFisher (granzyme B, Hs00188051_m1, amplification region exons 4–5; perforin, Hs00169473_ml, amplification region exons 2–3). Real-time PCR was performed on the IQ5 (Bio-Rad). Human GAPDH served as the normalizer. The relative gene expression was expressed as fold change calculated by the ΔΔCt method.

ELISA

Human gastric tissues from specimens were collected, homogenized in 1 ml sterile Protein Extraction Reagent, and centrifuged. Tissue supernatants were collected for ELISA. Concentrations of GM-CSF in the tissue supernatants were determined using ELISA kits according to the manufacturer's instructions. Proteins from mouse tumours were extracted using FFPE Total Protein Extraction Kit (Sangon Biotech) according to the manufacturer's instructions. Tumour tissue supernatants were collected for ELISA. Concentrations of perforin and granzyme B in the tumour tissue supernatants were determined using ELISA kits according to the manufacturer's instructions.

Western blot analysis

Western blot assays were performed on 10%-15% SDS-PAGE gels using equivalent amounts of cell lysate proteins of samples. Five percent skimmed milk or three percent BSA was used for blocking the PDF membranes. Human STAT3 and p-STAT3 were detected with rabbit anti-STAT3 and rabbit anti-p-STAT3 antibodies respectively. This was followed by incubation with HRP-conjugated secondary antibodies. Bound proteins were visualized by using SuperSignal® West Dura Extended Duration Substrate kit.

Microarray experiments

Gene expression profiles of human tumour tissues from GC patients were analyzed with the Affymetrix GeneChip Human Gene 1.0 ST Array (Affymetrix), strictly following the manufacturer's protocol. Microarray experiments were performed at the Genminix Informatics (China) with the microarray service certified by Affymetrix.