**Table S1. Antibody information**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Target | Manufacturer | Catalog  number | IHC  (dilution) | IF  (dilution) | Flow  cytometry  (dilution) |
| Primary | PDGFC | R&D Systems | AF1560 | 1:100 |  |  |
|  | PDGFD | R&D Systems | AF1159 | 1:100 |  |  |
|  | PDGFR | Cell Signaling  Technology | 5241 | 1:100 | 1:100 |  |
|  | PDGFR | Cell Signaling  Technology | 4564 | 1:100 | 1:100 |  |
|  | pan cytokeratin | Abcam | ab80826 |  | 1:100 |  |
|  | -SMA | LSBio | LS-B3933 |  | 1:200 |  |
|  | -SMA | Abcam | ab5694 |  | 1:100 |  |
|  | COL1A1 | Cell Signaling  Technology | 72026 |  | 1:100 |  |
|  | CD140a (PDGFR) | BioLegend | 135923 |  |  | 1:200 |
|  | CD140b (PDGFR) | BioLegend | 136007 |  |  | 1:200 |
|  | Ly6G | BioLegend | 127605 |  |  | 1:500 |
|  | CD45 | BioLegend | 103132 |  |  | 1:200 |
|  | Ly6C | BioLegend | 128015 |  |  | 1:200 |
|  | CD11b | BioLegend | 101262 |  |  | 1:200 |
|  | CD4 | BioLegend | 100510 |  |  | 1:500 |
|  | CD3 | BioLegend | 100328 |  |  | 1:200 |
|  | CD25 | BioLegend | 102008 |  |  | 1:200 |
|  | PD-1 | BioLegend | 135216 |  |  | 1:200 |
|  | IFNγ | BioLegend | 505826 |  |  | 1:200 |
|  | FOXP3 | eBiosceince | 25-5773-82 |  |  | 1:200 |
|  | CD8a | BioLegend | 100712 |  |  | 1:200 |
|  | CD45 | BioLegend | 103154 |  |  | 1:200 |
|  | NK1.1 | BioLegend | 108732 |  |  | 1:200 |
|  | CD90.2 | BioLegend | 105308 |  |  | 1:200 |
|  | EpCAM | BioLegend | 118216 |  |  | 1:200 |
| Secondary | Anti-rabbit  IgG-HRP | Dako | K4003 |  |  |  |
|  | Anti-goat IgG-HRP | Nichirei | 414162F |  |  |  |
|  | Alexa Fluor 488-conjugated donkey  anti-mouse IgG | Invitrogen | A21202 |  | 1:500 |  |
|  | Alexa Fluor 555-conjugated donkey  anti-goat IgG | Invitrogen | A21432 |  | 1:500 |  |
|  | Alexa Fluor 647-conjugated donkey  anti-rabbit IgG | Invitrogen | A31573 |  | 1:500 |  |

**Table S2. Primer/probe information**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Organism | Official Symbol | Alias | Forward primer | Reverse primer |
| Human | *ACTB* | β-actin | attggcaatgagcggtt | cgtggatgccacaggact |
|  | *PDGFRa* |  | ttcaatggacttaccctggag | atttgatggatgggactttga |
|  | *PDGFRb* |  | ctggtctggggccattag | tgggggtgtcctgtacttg |
|  | *CXCL3* |  | aaatcatcgaaaagatactgaacaag | ggtaagggcagggaccac |
|  | *CXCL5* |  | ggtccttcgagctccttgt | gcagctctctcaacacagca |
|  | *CXCL8* |  | agcacacaagcttctaggaca | ctgcaccttcacacagagct |
|  | *VEGFA* |  | cctccgaaaccatgaacttt | atgattctgccctcctcctt |
| Mouse | *Actb* | β-actin | ctaaggccaaccgtgaaaag | accagaggcatacagggaca |
|  | *Pdgfra* |  | gcaccaagtcaggtcccatt | tgtccaggtctttcttcggc |
|  | *Pdgfrb* |  | aaaccaccattggggacagg | cgttgatggatgacacctgga |
|  | *Pdgfc* |  | tgtgtcccacgtaaagttacaaa | tcagtgagtgacttatgcaatcc |
|  | *Pdgfd* |  | ctttgaccatcaattcggact | cttcaacttccacaaagtcatacc |
|  | *Tgfb1* |  | ccttcctgctcctcatgg | cgcacacagcagttcttctc |
|  | *Cxcl1* |  | actcaagaatggtcgcgagg | acttggggacaccttttagca |
|  | *Cxcl3* |  | ccccaggcttcagataatcat | aaagacacatccagacaccg |
|  | *Vegfa* |  | gcagcttgagttaaacgaacg | ggttcccgaaaccctgag |

**Table S3. Information on primary antibodies used for RPPA.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| # | Antigen | Antibody | Clone | Manufacturer | Catalog  number | Dilution |
| 1 | Phospho-MAPKAPK-2 (Thr334) | Rabbit monoclonal | 27B7 | Cell Signaling  Technology | 3007 | 1:1000 |
| 2 | Phospho-MAPKAPK-2 (Thr222) | Rabbit monoclonal | 9A7 | Cell Signaling  Technology | 3316 | 1:1000 |
| 3 | Phospho-p38 MAPK (Thr180/Y182) | Rabbit monoclonal | D3F9 | Cell Signaling  Technology | 4511 | 1:1000 |
| 4 | Phospho-Akt (Ser473) | Rabbit monoclonal | D9E | Cell Signaling  Technology | 4060 | 1:2000 |
| 5 | Phospho-S6 Ribosomal Protein (Ser235/236) | Rabbit monoclonal | D57.2.2E | Cell Signaling  Technology | 4858 | 1:2000 |
| 6 | Phospho-S6 Ribosomal Protein (Ser240/244) | Rabbit monoclonal | D68F8 | Cell Signaling  Technology | 5364 | 1:1000 |
| 7 | Phospho-mTOR (Ser2448) | Rabbit monoclonal | D9C2 | Cell Signaling  Technology | 5536 | 1:1000 |
| 8 | Phospho-70 S6 kinase (Thr389) | Rabbit monoclonal | 108D2 | Cell Signaling  Technology | 9234 | 1:1000 |
| 9 | Phospho-Akt (Thr308) (D25E6 | Rabbit monoclonal | D25E6 | Cell Signaling  Technology | 13038 | 1:1000 |
| 10 | Phospho-44/42 MAPK (Erk1/2) (Thr202/Tyr204) | Rabbit monoclonal | D13.14.4E | Cell Signaling  Technology | 4370 | 1:2000 |
| 11 | Phospho-MEK1/2 (Ser217/221) | Rabbit monoclonal | 41G9 | Cell Signaling  Technology | 9154 | 1:1000 |
| 12 | Phospho-SHC (Tyr317) | Rabbit polyclonal | N/A | Upstate | 206 | 1:1000 |
| 13 | Phospho-Src Family (Tyr416) | Rabbit polyclonal | N/A | Cell Signaling  Technology | 2101 | 1:1000 |
| 14 | Phospho-Src (Tyr527) | Rabbit polyclonal | N/A | Cell Signaling  Technology | 2105 | 1:1000 |
| 15 | Nonphospho-Src (Tyr527) | Rabbit polyclonal | N/A | Cell Signaling  Technology | 2107 | 1:1000 |
| 16 | Phospho-PDGFRβ (Thy1021) | Rabbit monoclonal | 6F10 | Cell Signaling  Technology | 2227 | 1:1000 |
| 17 | Phospho-PDGFRβ (Thy1009) | Rabbit monoclonal | 42F9 | Cell Signaling  Technology | 3124 | 1:1000 |
| 18 | Phospho-PDGFRβ (Thy751) | Rabbit monoclonal | C63G6 | Cell Signaling  Technology | 4549 | 1:1000 |
| 19 | Phospho-IGF-I Receptor β (Tyr1131)/Insulin Receptor β (Tyr1146) | Rabbit polyclonal | N/A | Cell Signaling  Technology | 3021 | 1:1000 |
| 20 | Phospho-IGF-I Receptor β(Tyr1135/1136)/Insulin Receptor β(Tyr1150/1151) | Rabbit monoclonal | 19H7 | Cell Signaling  Technology | 3024 | 1:1000 |
| 21 | Phospho-IGF-I Receptor β (Tyr980) | Rabbit monoclonal | C14A11 | Cell Signaling  Technology | 4568 | 1:1000 |
| 22 | Phospho-PLCγ (Tyr783) | Rabbit polyclonal | N/A | Cell Signaling  Technology | 2821 | 1:1000 |
| 23 | Phospho-PLCγ2 (Tyr1217) | Rabbit polyclonal | N/A | Cell Signaling  Technology | 3871 | 1:1000 |
| 24 | Phospho-PLCγ2(Tyr759) | Rabbit polyclonal | N/A | Cell Signaling  Technology | 3874 | 1:1000 |
| 25 | Phospho-c-Kit (Tyr703) | Rabbit monoclonal | D12E12 | Cell Signaling  Technology | 3073 | 1:1000 |
| 26 | Phospho-FGF Receptor (Tyr653/654) | Rabbit polyclonal | N/A | Cell Signaling  Technology | 3471 | 1:1000 |
| 27 | Phospho-VEGF Receptor 2 (Tyr1175) | Rabbit monoclonal | D5B11 | Cell Signaling  Technology | 3770 | 1:1000 |
| 28 | Phospho-VEGF Receptor 2 (Tyr1059) | Rabbit monoclonal | D5A6 | Cell Signaling  Technology | 3817 | 1:1000 |
| 29 | Phospho-VEGF Receptor 2 (Tyr951) | Rabbit monoclonal | 15D2 | Cell Signaling  Technology | 4991 | 1:1000 |
| 30 | Phospho-PDGF Receptor α　(Tyr1018) | Rabbit polyclonal | N/A | Cell Signaling  Technology | 4547 | 1:1000 |
| 31 | Phospho-PDGF Receptor α　(Tyr754) | Rabbit monoclonal | 23B2 | Cell Signaling  Technology | 2992 | 1:1000 |
| 32 | Src | Rabbit monoclonal | 36D10 | Cell Signaling  Technology | 2109 | 1:1000 |
| 33 | S6 Ribosomal Protein | Rabbit monoclonal | 5G10 | Cell Signaling  Technology | 2217 | 1:1000 |
| 34 | Shc | Rabbit polyclonal | N/A | Cell Signaling  Technology | 2432 | 1:1000 |
| 35 | p70 S6 Kinase | Rabbit monoclonal | 49D7 | Cell Signaling  Technology | 2708 | 1:1000 |
| 36 | PLCγ1 | Rabbit polyclonal | N/A | Cell Signaling  Technology | 2822 | 1:1000 |
| 37 | PLCγ2 | Rabbit polyclonal | N/A | Cell Signaling  Technology | 3872 | 1:1000 |
| 38 | mTOR | Rabbit monoclonal | 7C10 | Cell Signaling  Technology | 2983 | 1:1000 |
| 39 | MAPKAPK-2 | Rabbit polyclonal | N/A | Cell Signaling  Technology | 3042 | 1:1000 |
| 40 | PDGFRβ | Rabbit monoclonal | 28E1 | Cell Signaling  Technology | 3169 | 1:1000 |
| 41 | Akt (pan) | Rabbit monoclonal | C67E7 | Cell Signaling  Technology | 4691 | 1:1000 |
| 42 | p44/42 MAPK (Erk1/2) | Rabbit monoclonal | 137F5 | Cell Signaling  Technology | 4695 | 1:1000 |
| 43 | p38 MAPK | Rabbit monoclonal | D13E1 | Cell Signaling  Technology | 8690 | 1:1000 |
| 44 | MEK1/2 | Rabbit monoclonal | 47E6 | Cell Signaling  Technology | 9126 | 1:1000 |
| 45 | FGF Receptor 1 | Rabbit monoclonal | D8E4 | Cell Signaling  Technology | 9740 | 1:1000 |
| 46 | FGF Receptor 3 | Rabbit monoclonal | C51F2 | Cell Signaling  Technology | 4574 | 1:1000 |
| 47 | FGF Receptor 4 | Rabbit monoclonal | D3B12 | Cell Signaling  Technology | 8562 | 1:1000 |
| 48 | PDGFRα | Rabbit monoclonal | D13C6 | Cell Signaling  Technology | 5241 | 1:1000 |

**Table S4. Clinicopathological characteristics of patients with GC**

|  |  |  |  |
| --- | --- | --- | --- |
| Variables | Stroma-high group  (n=136) | Stroma-low group  (n=202) | p value |
| Age, Years  Median (IQR) | 69.5 (62.5–77.3) | 71.0 (62.3–78.8) | 0.28 |
| Sex  Male (%)  Female (%) | 99 (72.8)  37 (27.2) | 148 (73.3)  54 (26.7) | 1.00 |
| T stage  1, 2 (%)  3, 4 (%) | 41 (30.1)  95 (69.9) | 122 (60.4)  80 (39.6) | <0.001 |
| N stage  0 (%)  1-3 (%) | 59 (43.4)  77 (56.6) | 119 (58.9)  83 (41.1) | 0.006 |
| Venous invasion  positive (%) | 104 (76.5) | 108 (53.5) | <0.001 |
| Lymphatic invasion  positive (%) | 59 (43.4) | 77 (38.1) | 0.37 |
| Stage  I, II (%)  III, IV (%) | 78 (57.4)  58 (42.6) | 160 (79.2)  42 (20.8) | <0.001 |
| Recurrence after curative resection (%) | 45 (33.1) | 32 (15.8) | <0.001 |

**Table S5. Clinicopathological characteristics of patients with IGC**

|  |  |  |  |
| --- | --- | --- | --- |
| Variables | Stroma-high group  (n=75) | Stroma-low group  (n=126) | p value |
| Age, Years  Median (IQR) | 70.0 (63.5–78.0) | 73.0 (64.0–79.8) | 0.23 |
| Sex  Male (%)  Female (%) | 57 (76.0)  18 (24.0) | 101 (80.2)  25 (19.8) | 0.61 |
| T stage  1, 2 (%)  3, 4 (%) | 28 (37.3)  47 (62.7) | 82 (65.1)  44 (34.9) | <0.001 |
| N stage  0 (%)  1-3 (%) | 34 (45.3)  41 (54.7) | 81 (64.3)  45 (35.7) | 0.013 |
| Venous invasion  positive (%) | 56 (74.7) | 65 (51.6) | 0.003 |
| Lymphatic invasion  positive (%) | 29 (38.7) | 43 (34.1) | 0.68 |
| Stage  I, II (%)  III, IV (%) | 51 (68.0)  24 (32.0) | 107 (84.9)  19 (15.1) | 0.008 |
| Recurrence after curative resection (%) | 18 (24.0) | 20 (15.9) | 0.22 |

**Table S6. Clinicopathological characteristics of patients with DGC**

|  |  |  |  |
| --- | --- | --- | --- |
| Variables | Stroma-high group  (n=61) | Stroma-low group  (n=76) | p value |
| Age, Years  Median (IQR) | 68.0 (60.0–77.0) | 68.0 (60.0–76.3) | 0.99 |
| Sex  Male (%)  Female (%) | 42 (68.9)  19 (31.1) | 47 (61.8)  29 (38.2) | 0.47 |
| T stage  1, 2 (%)  3, 4 (%) | 13 (21.3)  48 (78.7) | 40 (52.6)  36 (47.4) | <0.001 |
| N stage  0 (%)  1-3 (%) | 25 (41.0)  36 (59.0) | 38 (50.0)  38 (50.0) | 0.31 |
| Venous invasion  positive (%) | 48 (78.7) | 43 (56.6) | 0.008 |
| Lymphatic invasion  positive (%) | 30 (49.2) | 34 (44.7) | 0.11 |
| Stage  I, II (%)  III, IV (%) | 27 (44.3)  34 (55.7) | 53 (69.7)  23 (30.3) | 0.003 |
| Recurrence after curative resection (%) | 27 (44.3) | 12 (15.8) | <0.001 |

**Supplementary Figure legends**

**Supplementary Figure 1 Assessment of the amount of stroma.**

(A) Workflow to evaluate the tumor stroma. (B) Comparison of OS between the stroma-high and stroma-low groups of all patients with GC in the Kumamoto cohort.

**Supplementary Figure 2 OS curves for patients with DGC and IGC from TCGA database based on their PDGF mRNA expression levels.**

(A) Comparison of OS between patients with DGC and IGC presenting PDGFC- or D-high tumors in the Kumamoto cohort. (B) Comparison of OS between patients with DGC and IGC presenting PDGFC- or D-low tumors in the Kumamoto cohort.

**Supplementary Figure 3 Chemotaxis assay of PMN-MDSCs.**

Chemotaxis of PMN-MDSCs using CM from cocultured fibroblasts treated with the anti-CXCL1 or anti-CXCL3 antibody. All data presented in this figure are shown as the means ± SD. n=3 replicates per group. \*\*p < 0.01 and \*\*\*p < 0.001.

**Supplementary Figure 4 The protocol of serial transplantation.**

KP cells (5.0×10⁵cells) were implanted subcutaneously into the flanks of eight- to ten-week-old male C57BL/6N mice. The mice were sacrificed 3 weeks after tumor cell inoculation. The tumor was dissociated and cultured for 1 week and then implanted subcutaneously into the mouse flank. The mice were sacrificed 3 weeks after tumor cell inoculation. This process was repeated as the serial transplantation method.

**Supplementary Figure 5 Evaluation of the amount of stroma in serially transplanted GAN-KP tumors.**

Representative images of Sirius red staining of S0, S3 and S5 tumors. Scale bars, 100 μm (upper panels) and 50 µm (lower panels).

**Supplementary Figure 6 Flow cytometry evaluation of cultured cells immediately before serial transplantation.**

A representative flow cytometry evaluation of the suspension of cultured cells immediately before serial transplantation after one week of in vitro culture. Forward- and side-scatter were used to eliminate debris, and 7AAD staining was performed to eliminate dead cells. CD90 was used as a fibroblast marker, and EpCAM was used as an epithelial cell marker.

**Supplementary Figure 7 The protocol for anti-PD-1 immunotherapy.**

S0 and S5 KP cells were subcutaneously transplanted into C57BL/6 mice. After 7 days, the mice were administered an anti-PD1 antibody (n=7) or IgG control (n=5) five times every other day. The mice were euthanized on day 21.

**Supplementary Figure 8 H&E staining and spatial clustering of the control group.**

H&E staining of the cryosectioned tumor from the control group (left panel) and UMAP embedding of all Visium spots from the two samples colored by cluster assignments (right panel). The clustering result was overlaid as colored spots on the images of H&E staining (middle panel). Scale bar, 2 mm.

**Supplementary Figure 9 Clustering analysis of cells within the tumor using cell-type specific canonical markers.**

UMAP plots show S5 KP cell subpopulations marked by gene modules specific for each subpopulation. Genes used to assign module scores are listed beneath each subpopulation.

**Supplementary Figure 10 Evaluation of PDGFRβ and COL1A1 expression in serially transplanted GAN-KP tumors after treatment.**

Immunofluorescence staining for PDGFRβ and COL1A1 and DAPI nuclear staining of serially transplanted GAN-KP tumors from animals administered the vehicle, anti-PD1 antibody, regorafenib or anti-PD1 antibody+regorafenib. The graph shows the quantification of the PDGFRβ- or COL1A1-positive areas. Data are presented as the means ± SD. Twelve dots in each group were randomly selected from 4 fields of view in each of the 3 samples. Scale bars, 100 μm.