

RESEARCH ARTICLE

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Spatial Transcriptomic Profiling of *FAP*-Positive Cells in Gastric Cancer and Association with Immunotherapy Efficacy

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Abstract

Introduction: The spatial heterogeneity of the tumor microenvironment in gastric cancer, particularly in the stromal compartment, remains poorly characterized. Fibroblast activation protein (FAP)-positive cancer-associated fibroblasts (CAFs) are known to promote tumor progression, immune evasion, and therapeutic resistance. However, the spatial transcriptional programs of *FAP*⁺ and *FAP*⁻ stroma and their associations with response to immunotherapy are not fully understood. **Material and methods:** Formalin-fixed, paraffin-embedded (FFPE) gastric cancer tissue sections were analyzed with the 10x Genomics Visium Spatial Gene Expression platform, and IHC was used to validate the transcriptional findings. **Results:** The *FAP*⁺ stromal regions displayed a transcriptional profile characterized by extensive extracellular matrix remodeling (*COL1A1*, *FNI*, *SPARC*), immunosuppressive signaling (*GREM1*, *CIS*, *IFI44*), and high expression of canonical CAF markers (*ACTA2*, *TAGLN*). In contrast, *FAP*⁻ regions retained epithelial features, indicating a closer proximity to tumor cells, and showed enrichment in antigen presentation and interferon signaling pathways. Pathway analysis confirmed the divergence in functional states between these stromal regions. Importantly, *FAP*⁺ regions in non-responders to immunotherapy exhibited overexpression of fibrosis- and stemness-associated genes, along with immunosuppressive chemokines (*CCL20/28*), whereas responders demonstrated robust interferon signaling, antigen processing, and epithelial differentiation. Expression of *CD24* in fibroblasts was validated by immunohistochemistry, confirming transcriptomic data and suggesting potential new drug targets in tumor stroma. **Conclusion:** *FAP*⁺ regions, in contrast to *FAP*⁻ clusters, exhibited dense fibrosis, distinct metabolic profiles, and altered immune signatures. Non-responders to immunotherapy displayed a fibrotic, immunosuppressive *FAP*⁺ signature associated with stemness and radioresistance, supporting the use of combined *FAP*-targeted radionuclide therapy with radiosensitizers. Conversely, responders' *FAP*⁺ niches showed immune activation and interferon signaling, favoring immunomodulatory treatments.

Keywords: gastric cancer, cancer-associated fibroblasts, spatial transcriptomics, FAP, tumor microenvironment

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Introduction

The advent of theranostics a paradigm integrating diagnostics and therapeutics has emerged as one of the most transformative advances in 21st-century oncology. While traditional approaches have focused on targeting cancer cells directly, recent breakthroughs have shifted toward exploiting the tumor microenvironment (TME), particularly stromal components that drive therapy resistance. Among these, fibroblast activation protein- α (FAP) has surfaced as a promising target, given its overexpression in cancer-associated fibroblasts (CAFs), which orchestrate immunosuppression, extracellular matrix (ECM) remodeling, and tumor progression.

Crucially, *FAP*-expressing cells in gastric cancer (GC) are heterogeneous. Recent studies reveal that *FAP*⁺ gastric

cancer mesenchymal stromal cells (GCMSCs) promote tumor progression via paracrine signaling (e.g., INHBA) and ECM remodeling [1]. Moreover, cross-talk between *FAP*⁺ fibroblasts and SPP1⁺ macrophages drives immune evasion in mismatch repair-proficient (pMMR) GC [2]. Preclinical evidence suggests that targeting *FAP*⁺ CAFs enhances the efficacy of immune checkpoint blockade (ICB) [3], while prognostic models incorporating CAF-secreted factors (e.g., GLT8D2, SPARC, VCAN) stratify GC patients into distinct risk groups [4]. Notably, GPC3^{high} CAFs have been implicated in resistance to PD-1 inhibition, and their ablation sensitizes tumors to immunotherapy [5].

These findings highlight the potential of combining *FAP*-targeted radionuclide therapy (TRT) with ICB. The term "radionuclide" in this context refers to the

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delivery of cytotoxic radiation directly to the tumor microenvironment. This process is not merely a parallel treatment but can actively potentiate immunotherapy through several key mechanisms. The radiation emitted by the radionuclide (e.g., Lutetium-177 or Yttrium-90) induces immunogenic cell death in both cancer cells and *FAP*-positive stromal cells. This releases tumor-associated antigens and molecular signals that act as an “in situ vaccine”, priming and recruiting tumor-specific T-cells. Moreover, by selectively targeting and depleting *FAP*⁺ CAFs, TRT can disrupt the physical and immunosuppressive barrier of the TME. This remodeling may enhance T-cell infiltration into the tumor core, a critical factor for the success of ICB. Preclinical models demonstrate that this TRT-induced immune activation and stromal disruption create a favorable environment for ICB, leading to synergistic antitumor effects [6]. Early clinical trials now support the feasibility and preliminary efficacy of this combined approach [7].

Here, we dissect the *FAP*-associated stromal landscape in gastric cancer, comparing patients with favorable versus poor responses to immunotherapy. By integrating transcriptomic profiling, spatial biology, and clinical outcomes, we identify stromal-immune mechanisms driving resistance to ICB.

Materials and Methods

Patient Cohort and Ethics

This study analyzed tissue samples from eight patients (n=8; 4 men, 4 women; mean age 61.0 ± 8.44 years) with histologically confirmed gastric adenocarcinoma (stages T2–4N0–1M0). Diagnosis followed the WHO Classification of Tumors of the Digestive System, 5th Edition (2019). Six patients had well-differentiated (grade 1) and two had poorly differentiated (grade 3) tumors. Clinical staging used the 8th edition AJCC/TNM system.

The study was approved by the Local Ethics Committee of Tomsk National Research Medical Center (Protocol No. 7, August 25, 2020) and complied with the Declaration of Helsinki. All participants gave written informed consent.

Immunohistochemistry Analysis of PD-L1 Expression

All patients underwent comprehensive diagnostic workup, including initial endoscopic biopsy, clinical staging, and immunohistochemical assessment of *PD-L1* expression. *PD-L1* status was evaluated using the Dako 22C3 pharmDx assay (Agilent Technologies, Santa Clara, CA, USA) with the Combined Positive Score (CPS) algorithm. Only patients with CPS >1 were included in the study.

Neoadjuvant Treatment

Patients underwent eight cycles of neoadjuvant chemotherapy using the FLOT regimen: docetaxel (50 mg/m² IV), oxaliplatin (85 mg/m² IV over 2 hours), leucovorin (200 mg/m² IV over 2 hours), and 5-fluorouracil (2600 mg/m² continuous IV infusion over 48 hours), repeated every 14 days. All also received pembrolizumab

(400 mg every 6 weeks). Clinical response was assessed before surgery using RECIST 1.1 criteria via contrast-enhanced CT scans at baseline and after 4 weeks. No serious adverse events causing treatment discontinuation were reported.

Surgical Treatment and Histopathological Evaluation

Radical surgery was performed 4 weeks after neoadjuvant therapy completion, with postoperative hospital stays of 7 to 10 days. One patient died early postoperatively due to colonic ulcer perforation and peritonitis. Biopsy and resected tissues underwent standard histopathological examination: fixation in 10% neutral buffered formalin, routine processing, paraffin embedding, and hematoxylin-eosin staining. Tumors were evaluated for differentiation, perineural and lymphovascular invasion, and compared to initial biopsies, following WHO criteria (5th Edition, 2019). Patients were classified as responders (TRG 1-2) or non-responders (TRG 3-5) based on the Mandard score.

Spatial Transcriptomics

Spatial transcriptomic profiling was performed on FFPE tissue sections using the 10x Genomics Visium Spatial Gene Expression platform. A detailed description of tissue preparation, library construction, and sequencing methodology is available in the original publication [8].

Bioinformatic Analysis

Raw FASTQ data were processed with Space Ranger v1.3, aligning reads to the GRCh38 genome. Gene counting, clustering, and annotation were performed using default tools. Cross-sample normalization and batch correction used spaceranger aggr. ROIs were manually annotated in Loupe Browser v8.1.2 based on morphology and marker expression, selecting spots with *FAP* expression >0 (Figure 1). Spatial segmentation retained stromal tissue spots, excluding necrosis, vasculature, and lymphoid structures.

Pseudobulk Expression Profiling and Cellular Deconvolution Analysis

Visium spatial transcriptomics raw count matrices were aggregated into 24 pseudobulk profiles by summing UMIs within manually annotated regions per sample: 8 *FAP*-positive, 8 *FAP*-negative, and 4 *FAP*-positive profiles stratified by therapeutic response. Profiles were normalized to transcripts to million (TPM). Cell type proportions were estimated using the EPIC package (v1.1.7) in R (v4.3.3) with its default immune-centric reference of seven cell types: B cells, CAFs, CD4⁺ and CD8⁺ T cells, endothelial cells, macrophages, and NK cells.

Differential Gene Expression and Enrichment Analyses

Differential gene expression analysis between manually annotated spatial clusters was performed in Loupe Browser (v8.1.2) using the built-in Wilcoxon rank-sum test with Benjamini–Hochberg correction for multiple testing. Genes with adjusted p-value < 0.05 were considered differentially expressed [9].

Gene Ontology (GO) enrichment analysis focusing on

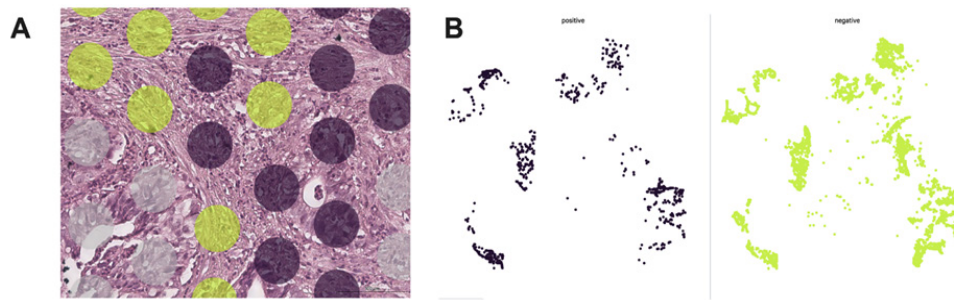


Figure 1. Spatial distribution of *FAP* expression in Gastric Cancer Tissue. Spots are color-coded: purple indicates no *FAP* expression; green indicates positive *FAP* expression; grey indicates spots without stromal cells or excluded because classified as tertiary lymphoid structure, necrosis, vessels or normal epithelium.

the Biological Process (BP) sub-ontology was performed in R (v. 4.4.2, <https://www.R-project.org/>) using the cluster Profiler (v3.10.0) [10] and enrich R (v3.4) packages [11]. DEG lists were used as input, and gene identifiers were converted to ENTREZ IDs using the org.Hs.eg.db (v3.21.0) annotation package. Enrichment was considered significant for GO terms with FDR < 0.05, adjusted using the Benjamini–Hochberg method. Enrichment results were visualized with ggplot2 (v3.5.0) [12], viridis (v0.6.5) [13], and enrichplot (v1.28.2) [14].

Immunohistochemistry for CD24

Validation of *CD24* expression differences was performed by immunohistochemistry (IHC). Formalin-fixed, paraffin-embedded (FFPE) tissue samples from the same patient cohort were stained using the Bond RXm automated system (Leica Biosystems, Germany) with anti-*CD24* primary antibody (1:100 dilution; Abclonal, China). Briefly, the slides were deparaffinized with Bond Dewax Solution, followed by antigen retrieval in Epitope Retrieval Solution 2 buffer (Leica Biosystems, Germany). Staining was carried out using the Bond Polymer Refine Detection Kit (Leica Biosystems, Germany), and hematoxylin counterstaining was applied. Images were captured using Aperio AT2 (Leica Biosystems, Germany), and the percentage of stained cells was quantified.

Statistical analysis

Statistical analyses were performed using Prism Graphpad 10. Pearson correlation analysis was used to assess the association between transcriptome analysis data and immunohistochemistry data. Differences with p-values < 0.05 were considered statistically significant.

Results

Differentially Expressed Genes in *FAP*+ versus *FAP*- Stroma in Gastric Cancer

Gastric cancer exhibits substantial heterogeneity not only at the epithelial level but also within the tumor stroma. CAFs, particularly those expressing fibroblast activation protein, contribute to tumor progression, immune evasion, and therapy resistance. However, the spatial and functional differences between *FAP*+ and *FAP*- stromal regions remain poorly characterized. To address this, we employed spatial transcriptomic profiling

to dissect gene expression landscapes in *FAP*+ versus *FAP*- stromal areas within gastric tumors. This approach enabled the identification of distinct molecular pathways, stromal remodeling signatures, and immunoregulatory features associated with *FAP*+ fibroblasts that may have prognostic and therapeutic implications. Comparative transcriptomic analysis of tumor regions containing *FAP*+ stromal cells versus *FAP*- cells revealed key molecular signatures indicative of active inflammatory processes and stromal remodeling (Table 1).

The *FAP*+ stromal niche in gastric cancer exhibits a profoundly remodeled extracellular matrix and immunosuppressive microenvironment. This fibrotic landscape is characterized by marked overexpression of structural collagens (*COL1A1/2*, *COL3A1*, *COL5A1*, *COL6A2/3*) and ECM glycoproteins (*FBN1*, *FN1*, *LUM*, *SPARC*), which drive desmoplastic stroma formation. Concurrent upregulation of matrix metalloproteinases (*MMP2*, *MMP14*) and their inhibitors (*TIMP1*, *TIMP3*) reflects dynamic ECM turnover. Immune processes in *FAP*+ regions are characterized by complement system inhibitors (*CD46*, *C1S*, *C1R*, *C3*), stromal-derived immunomodulators (*GREM1*, *THBS1*, *A2M*), and interferon-stimulated genes (*IFI44*, *IFI6*, *ISG15*, *OAS2*,

Table 1. Top 50 Differentially Expressed Genes in *FAP*+ vs. *FAP*- Stroma in Gastric Cancer

Biological Process	<i>FAP</i> + Stroma Signature Genes	<i>FAP</i> - Stroma Signature Genes
ECM Remodeling & Fibrosis	<i>COL1A1</i> , <i>COL1A2</i> , <i>COL3A1</i> , <i>COL5A1</i> , <i>FBN1</i> , <i>FN1</i> , <i>LUM</i> , <i>MMP2</i> , <i>TIMP1</i> , <i>TIMP3</i>	<i>MMP3</i>
Immune Modulation	<i>CD46</i> , <i>C1S</i> , <i>C1R</i> , <i>A2M</i>	<i>HLA-A</i> , <i>IGHA1</i> , <i>IGHJ6</i> , <i>LCN2</i> , <i>LAPTM5</i> , <i>TYROBP</i> , <i>CTSE</i> , <i>APOE</i>
Growth Factor Signaling	<i>PMEPA1</i> , <i>FSTL1</i> , <i>BGN</i>	<i>PLAUR</i> , <i>ITGA3</i>
Metabolic Pathways	<i>SCD</i> , <i>SOD3</i>	<i>FABP1</i> , <i>ALDOB</i> , <i>NAMPT</i> , <i>G0S2</i> , <i>DUOX2</i>
Interferon Response	<i>IFI44</i>	-
Prognostic Markers	<i>GREM1</i> , <i>TAGLN</i> , <i>ACTA2</i>	<i>PSCA</i> , <i>GOLM1</i> , <i>SPINK1</i>
Epithelial Features	-	<i>MUC5AC</i> , <i>TFF1</i> , <i>CLDN4</i> , <i>GKN1</i> , <i>MUC13</i> , <i>DMBT1</i>
Oxidative Stress	<i>SOD3</i>	<i>DUOX2</i> , <i>MT1G</i>

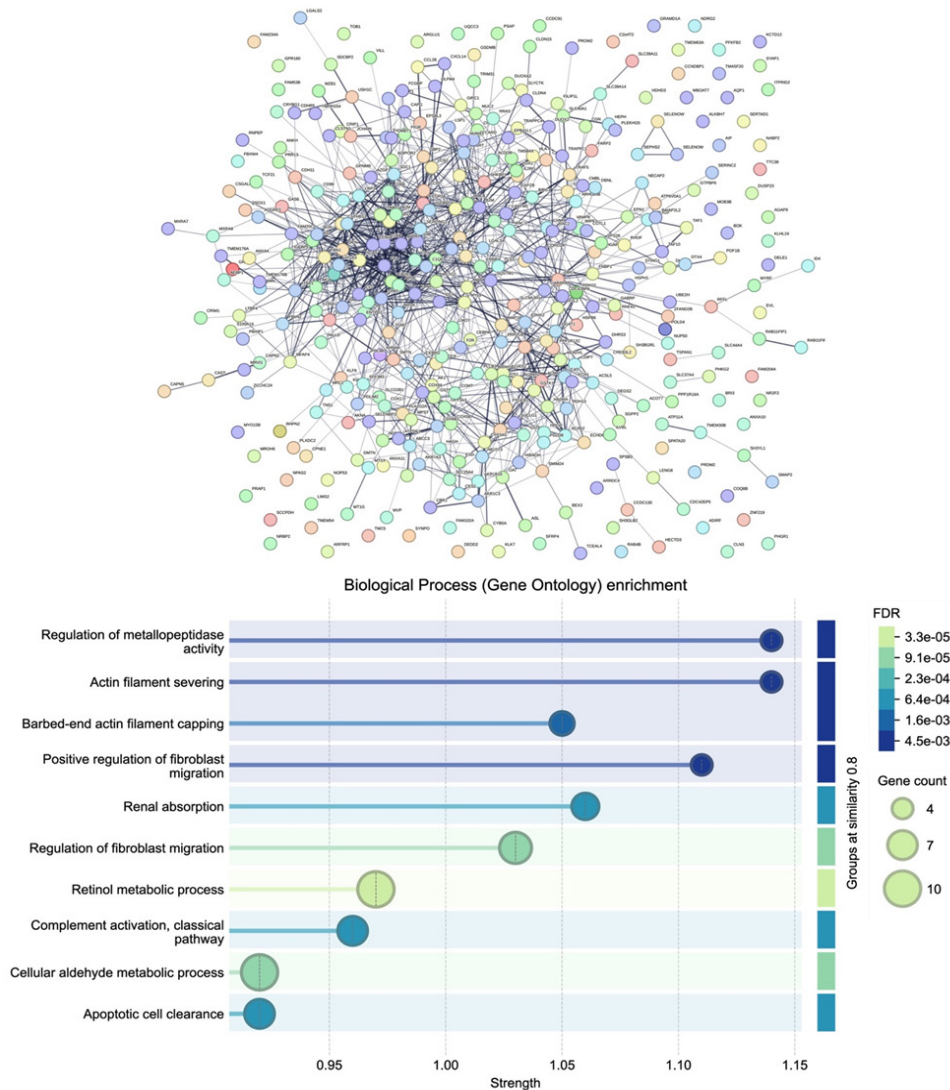


Figure 2. Transcriptomic Differences in *FAP*⁺ Stromal Regions in Gastric Cancer Patients with Poor Response to Immunotherapy

STAT1/2). Among the identified genes, several well-established CAF markers were particularly prominent, including *SPARC*, *TAGLN*, *ACTA2*, *VIM*, and *TNC*.

Unlike the *FAP*⁺ regions, *FAP*⁻ regions maintain epithelial integrity through the expression of gastric mucosal protectors (*TFF1*, *TFF2*, *MUC5AC*, *GKN1*) and tight junction proteins (*CLDN4*, *CLDN7*). *FAP*⁻ spots also contained markers of epithelial identity (*ELF3*, *KRT8*, *KRT7*). The immune profile of *FAP*⁻ regions was defined by coordinated expression of genes involved in antigen presentation (HLA-A, TAPBP), IFN- γ response (IFNGR2), cytokine signaling (*CXCL5*, *IL32*), complement activation (C1QC), and immunoglobulin production (*IGHA1*, *IGHM*, *IGLC1*).

Subsequent pathway analysis (Table 2) confirmed the enrichment of these immune-related biological processes.

Analysis of Overexpressed Genes in *FAP*⁺ Spots in Relation to Immunotherapy Response It was critically important to analyze how *FAP*⁺ stromal regions differ in gastric cancer patients according to their clinical response to immunotherapy.

To visualize these transcriptomic alterations associated with treatment outcomes, Figures 2 and 3 illustrate the differential gene expression in *FAP*⁺ stromal regions stratified by immunotherapy response.

Patients exhibiting poor response demonstrated hyperexpression of HLA-C, HLA-A, B2M, CD55, and FCGR2. These patients exhibited strong upregulation of fibrogenesis genes (*FAP*, *BGN*, *FN1*, *FBN1*, *TIMP3*, *LTBP1/4*, *CAPN2/5*), along with overexpression of *ALDH1A1/ALDH1A3*, *CEBPA*, and *TCF7L2*. They also showed elevated expression of stemness marker ID4, intestinal differentiation factor *CDX1*, and mesenchymal markers *VIM*, *TAGLN*, and *ACTG2*. The immunosuppressive signaling in these patients was mediated by chemokines *CCL20* and *CCL28*, which recruit regulatory T cells and myeloid-derived suppressor cells, while GAS6 promoted T-cell exhaustion.

In stark contrast to the immunosuppressive, fibrotic *FAP*⁺ stroma, gastric cancer patients who responded favorably to immunotherapy demonstrated robust expression of MHC class II molecules (*HLA-DRB1*,

Table 2. Biological Processes Significantly Enriched in FAP+ vs. FAP- Spots in Gastric Cancer

Name	P-value	Adjusted p-value	Odds Ratio	Combined score
FAP+ vs FAP-				
1 Collagen-Containing Extracellular Matrix (GO:0062023)	1.01E-24	1.71E-22	12.97	716.41
2 Focal Adhesion (GO:0005925)	7.20E-07	0.00002288	4.93	69.77
3 MHC Protein Complex (GO:0042611)	9.13E-07	0.00002288	36.46	507.08
4 Cell-Substrate Junction (GO:0030055)	9.42E-07	0.00002288	4.83	66.97
5 Basement Membrane (GO:0005604)	0.00000675	0.0001148	14.98	178.32
FAP-vs FAP+				
1 Oxidative Phosphorylation (GO:0006119)	2.88E-13	5.56E-10	20.34	587.23
2 Aerobic Electron Transport Chain (GO:0019646)	7.53E-13	6.09E-10	18.71	522.19
3 Mitochondrial ATP Synthesis Coupled Electron Transport (GO:0042775)	9.46E-13	6.09E-10	18.34	507.73
4 Cellular Respiration (GO:0045333)	3.15E-12	1.52E-09	14.35	380.04
5 Proton Motive Force-Driven Mitochondrial ATP Synthesis (GO:0042776)	1.71E-11	6.60E-09	19.92	493.79
Proton Motive Force-Driven Mitochondrial ATP Synthesis (GO:0042776)				

HLA-DRB5, *HLA-DQA1*, *HLA-F*) and antigen-processing machinery (*TAP1/2*), epithelial differentiation markers (*CD24*, *CD164*, *CEACAM5*), strong type I/II interferon signatures (*IFI44L*, *IFI44*, *IFI6*, *ISG15*, *OAS1-3*,

MX1/2), viral defense genes (*BST2*, *GBP5*, *DDX60*), and chemokines (*CXCL2*, *CXCL5*, *CXCL8*, *CXCL14*).

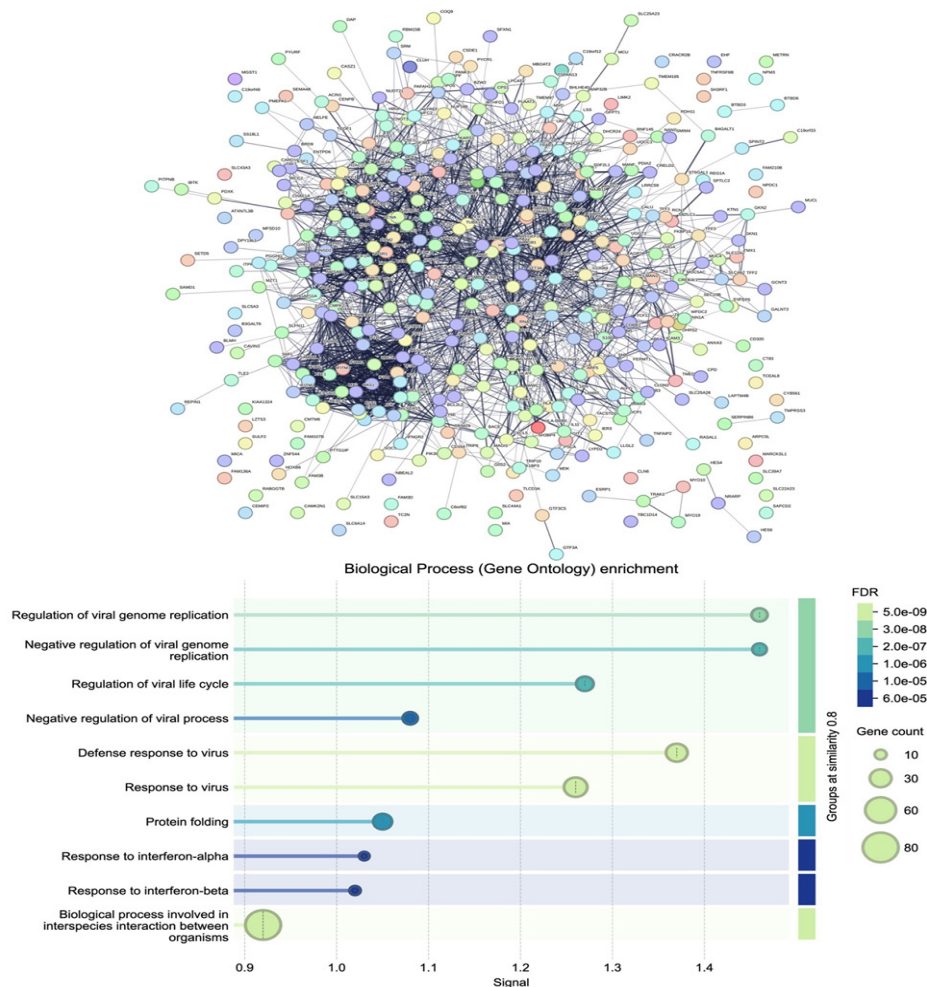


Figure 3. Transcriptomic Differences in FAP+ Stromal Regions in Gastric Cancer Patients with Good Response to Immunotherapy

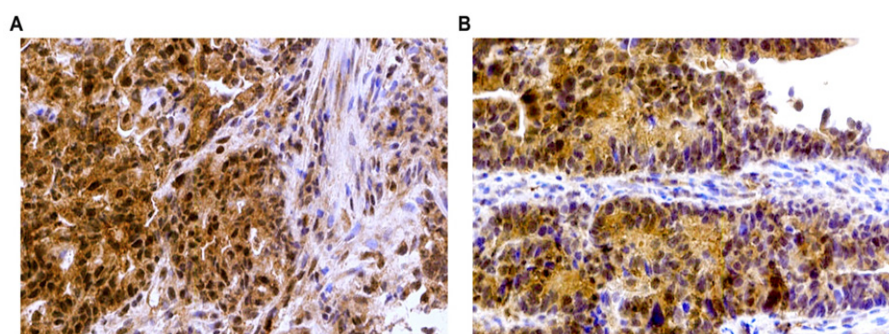


Figure 4. Immunohistochemical Characterization of *CD24* protein Expression Patterns in Fibroblast in Responders (A) and non-responders (B) to chemoimmunotherapy in gastric cancer. Magnification 630x.

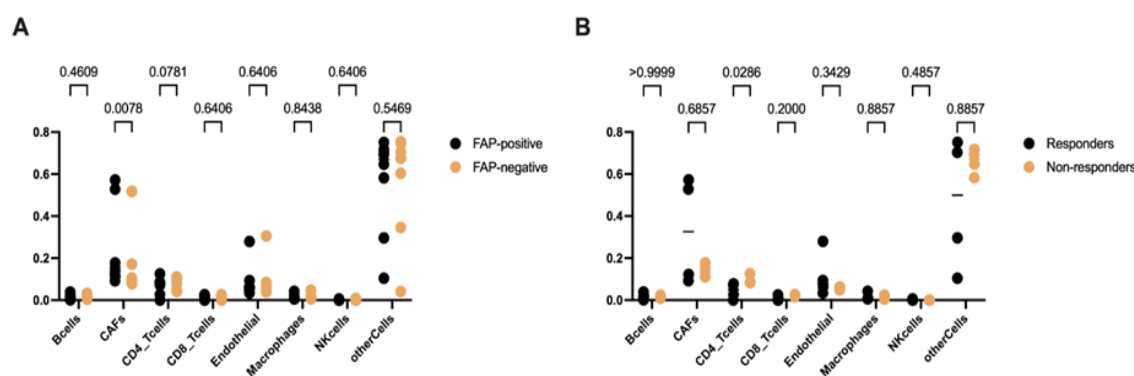


Figure 5. Cellular Deconvolution Analysis of Regions based on *FAP* Expression (A) and comparison of *FAP*+ regions between responders and non-responders to chemoimmunotherapy (B) in gastric cancer.

Validation of *CD24* Gene Expression Differences at the Protein Level Using IHC

To assess the consistency between *CD24* mRNA level in *FAP*+ stromal spots and protein expression of *CD24* in fibroblasts, we performed immunohistochemical analysis on clinical specimens (Figure 4). We assessed the percentage of *CD24*-positive fibroblasts within the stromal compartment. Notably, we observed pronounced nuclear localization of *CD24* in a subset of stromal cells, a finding that may have important functional implications.

For correlation analysis, we compared gene expression values with the percentage of *CD24*-positive cells in each sample. The results revealed a strong concordance between mRNA and protein levels, with *CD24* demonstrating a significant positive correlation ($R^2 = 0.88$, $p = 0.006$). This high correlation confirms the reliability of our transcriptomic data and validates *CD24* as a robust biomarker detectable at the protein level in clinical samples.

Cellular Composition of *FAP*- and *FAP*+ Regions in Patients with Gastric Cancer

Deconvolution analysis based on *FAP* expression revealed significant differences in the abundance of tumor-associated fibroblasts, which were more prevalent in *FAP*+ compartments (0.13 [0.11–0.44] relative units) compared to *FAP*- compartments (0.09 [0.08–0.44] relative units; $p = 0.0078$) (Figure 5A). Further comparison of the cellular composition between responders and non-responders to immunotherapy identified specific

cell types characterizing the microenvironment of *FAP*+ compartments. Notably, the level of *CD4*+ T cells was significantly higher in non-responders than in responders (0.08 [0.08–0.11] relative units vs. 0.04 [0.01–0.07] relative units; $p = 0.0286$) (Figure 5B).

Discussion

Recent studies investigating the molecular characteristics of tumor microenvironment cells are opening new avenues for the application of radionuclide therapy (RNT) in cancer treatment. Our study provided a detailed spatial characterization of *FAP*+ cells in gastric cancer tissue and highlighted their association with immunotherapy response.

We identified fundamental differences between *FAP*+ and *FAP*- niches within the GC microenvironment, which is of considerable importance for understanding tumor biology and developing novel therapeutic strategies. *FAP*+ stromal regions demonstrated pronounced extracellular matrix remodeling, with overexpression of structural collagens and glycoproteins contributing to a dense desmoplastic stroma. A hallmark of *FAP*+ niches was the presence of an immunosuppressive milieu, characterized by the expression of complement inhibitors, stromal immunomodulators, and interferon pathway genes. Several of the *FAP*+ associated genes identified in our study have previously been shown to have strong prognostic relevance in gastric cancer. For instance, Kitaoka et al. reported that the *GREM1*high

expression group was significantly associated with a more advanced pT grade, pN grade, lymphatic invasion, and venous invasion [15]. Subsequent findings highlighted the significance of GREM1+ fibroblast colocalization with SPP1+ macrophages. Qiu et al. demonstrated that patients with high level of both GREM1+ fibroblasts and SPP1+ macrophages exhibited significantly shorter overall survival and enrichment of tumor-associated pathways [16]. In another study, Yu et al. [17] reported that TAGLN levels were elevated in human gastric cancer stroma compared to normal gastric tissue and were associated with tumor differentiation and lymph node metastasis. Another gene of interest, *ACTA2*, appears to be linked not only to prognosis but also to the response to immunotherapy. Specifically, *ACTA2* expression in CAFs, but not in other cellular compartments, was associated with immune checkpoint inhibitor (ICI) responsiveness [18]. Cellular composition analysis also confirmed that *FAP*+ niches contained higher amounts of CAFs. Our findings demonstrate that prognostically relevant fibroblasts are specifically localized within *FAP*+ niches, emphasizing the high potential of *FAP*-targeted RNT as a precision approach in gastric cancer.

In contrast, *FAP*- regions were characterized by the expression of epithelial markers (*TFF1*, *TFF2*, *MUC5AC*, *GKN1*) and tight junction proteins (*CLDN4*, *CLDN7*). Although the spot selection criteria allowed for the presence of both stromal and tumor cells, *FAP*- spots predominantly exhibited markers of epithelial identity. This suggests that *FAP*+ fibroblasts are likely spatially separated from tumor cell clusters, or that tumor cells within *FAP*- regions display distinct transcriptomic profiles.

Importantly, *FAP*+ spots differed significantly in their molecular features depending on the patient's response to therapy. Patients who exhibited resistance to immunotherapy appear to be ideal candidates for RNT. Their tumors displayed dense fibrotic matrices with upregulation of *BGN*, *FNI*, and *COL1A1*, which may support prolonged retention of radiopharmaceuticals and thereby enhance therapeutic efficacy. High *FAP* expression itself further validates these regions as promising targets for radioligands such as $^{68}\text{Ga}/^{177}\text{Lu}$ -FAPI. However, these resistant tumors also expressed genes associated with radioresistance, including *ALDH1A3* and *AKR1B10*, suggesting the potential need for combined treatment strategies employing radiosensitizers [19, 20]. For such patients, an intensified treatment protocol may be developed using high-activity RNT (e.g., ^{177}Lu -FAPI at 7.4 GBq every 6–8 weeks). Analysis of the cellular composition of *FAP*+ spots in non-responders revealed a significant predominance of T-cells compared to responders. This finding aligns with the observed overexpression of chemokine genes *CCL20* and *CCL28*, which are known to recruit regulatory T-cells, thereby underscoring the critical role of regulatory T-cells in immunotherapy resistance, as we have previously demonstrated [21].

Conversely, in patients demonstrating favorable responses to immunotherapy, *FAP*+ areas exhibited fundamentally different characteristics: moderate *FAP*

expression, preserved vascularization, an active interferon response, and an overall immunoactive phenotype. In such cases, RNT may serve primarily as an immunomodulatory tool. A low-dose RNT protocol combined with concurrent immunotherapy may induce synergistic effects. A fractionated radiation schedule (2–3 cycles) may help maximize the immunostimulatory potential of radiation while minimizing collateral damage.

Among the numerous genes overexpressed in *FAP*+ regions of patients exhibiting a favorable response, we found it particularly interesting to validate *CD24* expression. Our data provide the first evidence of a positive association between *CD24* expression and response to immunotherapy, although it should be noted that existing data on this relationship remain limited. Notably, the TIDE database indicates that tumors with high *CD24* expression, especially melanoma, tend to be less responsive to *PD-1/PD-L1* immunotherapy [22].

CD24 expressed in fibroblast-like stromal cells from fetal liver but not in adult and fetal skin fibroblast [23]. Other study demonstrates that nuclear *CD24* induces *IL-6* expression by fibroblasts in vitro [24]. Although *IL-6* is traditionally regarded as immunosuppressive and pro-tumorigenic [25], emerging evidence suggests that it may also play a role in supporting anti-tumor immunity. Thus, it has been shown that blockade of the *IL-6* receptor inhibits the development of T-cells induced by a *TGF- β* -based vaccine, as well as the infiltration of T-cells into tumors. [26]. Our study provides indirect evidence for a potential mechanism by which fibroblasts contribute to a favorable response to immunotherapy and highlights the significance of the intracellular localization of *CD24* expression. It is important to note that the relevance of *CD24* localization has been addressed in previous studies. Thus, under drug treatment, translocation of *CD24* to the cell membrane is associated with sustained and strong p38 activation, as well as increased Bcl-2 expression, indicating a phenotype switch [27].

In conclusion, spatial transcriptomics of gastric cancer revealed key molecular and microenvironmental differences between *FAP*+ and *FAP*- tumor areas. *FAP*+ regions showed dense fibrosis, unique metabolic profiles, and altered immune gene expression influencing tumor behavior and therapy response. Poor immunotherapy responders had a *FAP*+ signature linked to fibrosis, stemness, immunosuppression, and radioresistance, supporting *FAP*-targeted radionuclide therapy combined with radiosensitizers. Responders' *FAP*+ niches showed immune activation and interferon signaling, favoring immunomodulatory treatments. These results demonstrate spatial transcriptomics' value for precision oncology and call for clinical trials of personalized radionuclide-immunotherapy based on these biomarkers.

Author Contribution Statement

L.T. - conception and design, drafting, A.K., E.S., E.T. - data acquisition, S.V. - resources/materials, R.Z. - funding acquisition, E.S., I.L. - critical review/editing.

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Ethical statement

The study was approved by the Local Ethics Committee of Tomsk National Research Medical Center (Protocol No. 7, August 25, 2020) and complied with the Declaration of Helsinki. All participants gave written informed consent.

Conflict of interest

The authors declare no conflict of interest.

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