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LETTER TO THE EDITOR

Single-cell and bulk transcriptomics identifies a tumor-specific CD36$^+$ cancer-associated fibroblast subpopulation in colorectal cancer

Dear Editor,

Cancer-associated fibroblasts (CAFs) are highly versatile and plastic cells in the tumor microenvironment. They have been identified as actively involved in cancer progression and metastasis through their various roles in remodeling the extracellular matrix, suppressing the immune response and reprogramming tumor metabolism [1]. However, many challenges exist in revealing the functional phenotypes and mechanisms of CAFs in different cancers due to limited understanding of CAF heterogeneity [2]. Recent advances in single-cell transcriptome technology have enabled the identification of distinct CAF subpopulations by using unique gene signatures in multiple tumor types [2]. In this study, we successfully identified a tumor-specific CD36$^+$ CAF subpopulation in colorectal cancer (CRC), which was found to be correlated with the number of tumor-infiltrated immune cells.

Primary CAFs and normal fibroblasts (NFs) were isolated from 5 fresh CRC tissues and paired normal colon tissues. Isolation methods and descriptions of other assays are shown in the Supplementary Methods. Immunofluorescence and Western blotting assays showed that the mesenchymal marker Vimentin was expressed in both NFs and CAFs, while alpha smooth muscle actin (αSMA) and fibroblast-specific protein 1 (FSP1) were overexpressed in CAFs (Supplementary Figure S1A-D). To further investigate gene expression profiles in these fibroblasts, primary NFs and CAFs were subjected to RNA sequencing. The results showed that CD36 was significantly upregulated in CAFs (Supplementary Figure S1E), which was further validated by immunofluorescence and Western blotting assays (Supplementary Figure S1F-G). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses of differentially expressed genes between NFs and CAFs indicated significant enrichment in the mitogen-activated protein kinase (MAPK) signaling pathway, phosphatidylinositol 3-kinase (PI3K)-Akt signaling pathway and receptor-ligand activity (Supplementary Figure S1H-I), suggesting that CAFs may play a critical role in intracellular and extracellular signal transduction.

Previous studies have demonstrated that CAFs achieve high heterogeneity and plasticity across different cancer types [1, 2]. To further reveal the characteristics of CAFs in CRC, we performed single-cell RNA-sequencing (scRNA-seq) using isolated NFs and CAFs and detected 21,248 NFs and 18,097 CAFs (Figure 1A). A total of 7 clusters were identified in these fibroblasts by using sample integration analysis based on distinct gene expression signatures. Interestingly, we found that Clusters 3, 5, and 7 were mainly distributed in the CAF group, accounting for 34.5%, 7.7%, and 2.3% of total CAFs, respectively (Figure 1B-E). Cluster gene signature analysis showed that CD36 was expressed in CAF-specific subgroups, such as Clusters 3 and 5; inhibin subunit beta A (INHBA) was mainly expressed in Clusters 3, 5 and 7; whereas fibroblast growth factor 7 (FGF7) and alcohol dehydrogenase 1B (ADH1B) were mainly expressed in Clusters 1 and 2, which made up the majority of NFs (Figure 1F). To further visualize the marker genes of CAFs, we presented single gene expression data by uniform manifold approximation and

Abbreviations: αSMA, Alpha smooth muscle actin; ACTA2, Actin alpha 2; ADH1B, Alcohol dehydrogenase 1B; CAFs, Cancer-associated fibroblasts; CXCL12, C-X-C motif chemokine ligand 12; FAP, Fibroblast activation protein; FGF7, Fibroblast growth factor 7; FN1, Fibronectin 1; FOXS1, Forkhead box S1; FPKM, Fragments per kilobase million; FSP1, Fibroblast-specific protein 1; GO, Gene Ontology; INHBA, Inhibin subunit beta A; KEGG, Kyoto Encyclopedia of Genes and Genomes; KRT18, Keratin 18; LTBP1, Latent transforming growth factor β-binding protein 1; MAPK, Mitogen-activated protein kinase; MFI, Mean fluorescence intensity; mIHC, Multiplex immunohistochemistry; NFs, Normal fibroblasts; PBS, Phosphate buffer saline; PI3K, Phosphatidylinositol 3-kinase; POSTN, Periostin; scRNA-seq, Single-cell RNA-sequencing; TCGA-COAD, The Cancer Genome Atlas-Colon Adenocarcinoma; THBS2, Thrombospondin 2; TIM-3, T-cell immunoglobulin domain and mucin domain-3; TNFSF4, Tumor necrosis factor ligand superfamily member 4; UMAP, Uniform manifold approximation and projection; UMI, Unique molecular identifiers.

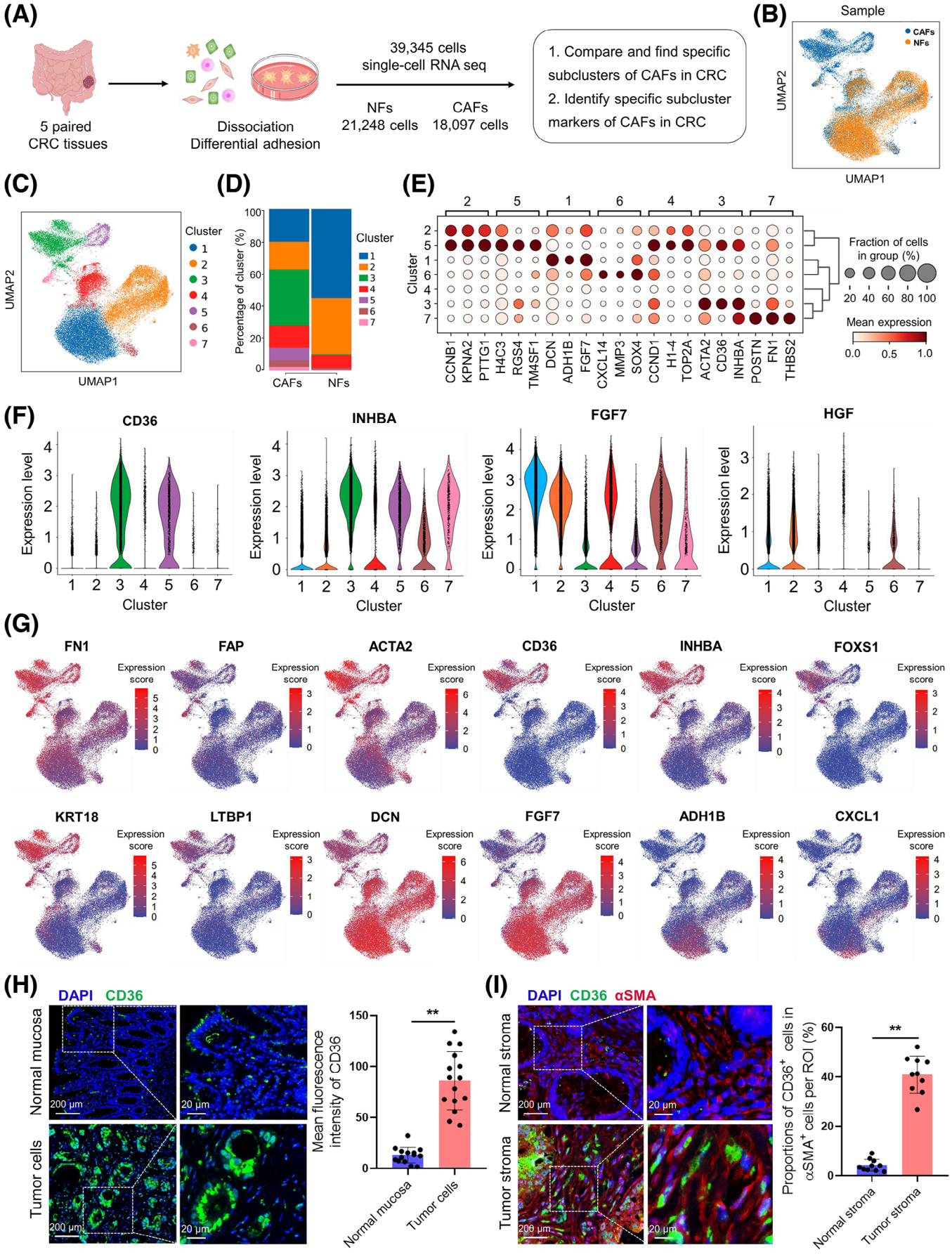

FIGURE 1 scRNA-seq identifies a tumor-specific CAF subpopulation in CRC. (A) Sample preparation diagram of scRNA-seq. (B, C) UMAP of distinct samples and fibroblast clusters. (D) Proportion of clusters in different samples. (E) Bubble plot depicting significant gene signatures in different clusters. (F) Violin plot of CD36, INHBA, FGF7, ADH1B in all fibroblast clusters. (G) UMAPs of single gene expression. (H) Representative immunofluorescence images of CD36 in 15 paired CRC tissues and adjacent normal tissues. The quantitative analysis of MFI was calculated by using Image J. (I) mIHC staining of CD36 and αSMA in 10 paired CRC tissues and adjacent normal tissues. The quantitative analysis of MFI was calculated using Image J.

Abbreviations: ACTA2, actin alpha 2; ADH1B, alcohol dehydrogenase 1B; aSMA, alpha smooth muscle actin; CAFs, cancer-associated fibroblasts; CXCL1, C-X-C motif chemokine ligand 12; DCN, decorin; FAP, fibroblast activation protein; FDF7, fibroblast growth factor 7; FN1, fibronectin 1; FOXS1, forkhead box S1; INHBA, inhibin subunit beta A; KAT18, keratin 18; LTBP1, latent transforming growth factor β binding protein 1; MFI, mean fluorescence intensity; mIHC, multiplex immunohistochemistry; NFs, normal fibroblasts; ROI, region of interest; scRNA-seq, single-cell RNA-sequencing; UMAP, uniform manifold approximation and projection.

projection (UMAP) and found that classical CAF markers, such as fibronectin 1 (FN1), fibroblast activation protein (FAP) and actin alpha 2 (ACTA2), cannot differentiate NFs and CAFs well, whereas a gene expression panel composed of CD36, INHBA, forkhead box S1 (FOXS1), keratin 18 (KRT18) and latent transforming growth factor β-binding protein 1 (LTBP1) was specifically expressed in CAFs (Figure 1G), suggesting the possibility that these genes could serve as new CAF markers in CRC.

To confirm the scRNA-seq results, we conducted multiplex immunohistochemistry (mIHC) staining and found that CD36 was overexpressed in both tumor cells and tumor stroma, especially in αSMA$^+$ fibroblasts (Figure 1H-I). CD36 staining using a CRC tissue microarray showed similar results (Supplementary Figure S2A-B). Survival analysis showed that higher expression of CD36 in tumor cells and stromal cells indicated poorer prognosis in CRC patients (Supplementary Figure S2C). In addition, we also performed survival analyses using gene expression data from The Cancer Genome Atlas-Colon Adenocarcinoma (TCGA-COAD) cohort and found that a panel of CAF marker genes [INHBA, FOXS1, periostin (POSTN), thrombospondin 2 (THBS2)] also indicated a poor prognosis in CRC patients (Supplementary Figure S2D-E).

Overall, we identified a CD36$^+$ CAF subpopulation in CRC and suggested the potential of CD36 as a specific CAF marker. Moreover, the survival analysis indicated that CAF marker genes (CD36, INHBA, FOXS1, POSTN, THBS2) may be promising prognostic indicators for CRC patients. Similarly, some previous studies have also indicated that CD36$^+$ CAFs and INHBA$^+$ CAFs may be predictors of a poor prognosis for hepatocellular carcinoma (HCC) [3] and gastric cancer patients [4], respectively. Mechanically, CD36$^+$ CAFs promote the formation of an immunosuppressive microenvironment in HCC [3]. To further investigate whether CD36 and INHBA are involved in immune microenvironment modulation in CRC, we analyzed gene expression data from the TCGA-COAD cohort and found that the expression of CD36 and INHBA was positively correlated with the expression of immunosuppressive factors and protumorigenic immune cell markers, such as C-X-C motif chemokine ligand 12 (CXCL12), tumor necrosis factor ligand superfamily member 4 (TNFSF4), T-cell immunoglobulin domain and mucin domain-3 (TIM-3) and CD163 (Supplementary Figure S3A-B). In agreement, estimation of the immune cell infiltration using the CIBERSORT method showed a significant positive correlation between M2 macrophage infiltration and CD36 expression as well as a significant negative correlation between CD8$^+$ T cell infiltration and INHBA expression (Supplementary Figure S3C-H). Based on these findings, we performed mIHC staining using CRC tissues and found that the infiltration of CD8$^+$ T cells in CD36$^+$ CAF-rich regions was significantly reduced (Supplementary Figure S4A-B), while the infiltration of CD68$^+$ CD163$^+$ macrophages in CD36$^+$ CAF-rich regions was increased (Supplementary Figure S4C-D).

CD36, a scavenger receptor expressed in tumor cells [5], regulatory T cells [6], CD8$^+$ T cells [7], and cancer-associated macrophages [8], promotes tumor metastasis and induces an immunosuppressive phenotype in different cancer types [5–8]. Tumor cells with elevated CD36 expression exhibit a unique ability to initiate metastasis, and the presence of CD36$^+$ metastasis-initiating cells associates with a poor prognosis of numerous cancers [9]. However, a previous study demonstrated that CD36 repressed colorectal tumorigenesis by inhibiting glycolysis and that its expression was gradually reduced from adenomas to carcinomas [10]. In our study, we identified that CD36 was overexpressed both in CRC cells and tumor stroma by using multiple methods, and we revealed that high expression of CD36 in CRC indicated poor patient outcomes. In addition, we found that the presence of CD36$^+$ CAFs was associated with decreased CD8$^+$ T cell infiltration and increased CD68$^+$ CD163$^+$ macrophage infiltration in CRC, suggesting that CD36$^+$ CAFs were associated with the suppressive immune phenotype. However, whether and how CD36$^+$ CAFs affect these immune cells remains to be further investigated.

Overall, CD36$^+$ CAFs are a poor prognostic factor in CRC and are associated with immune cell infiltration. Our work highlights the importance of identifying

tumor-specific CAF subpopulations in understanding the heterogeneous tumor microenvironment.

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CONFLICTS OF INTEREST STATEMENT

The authors disclose no conflicts.

CONTENT FOR PUBLICATION

Not applicable.

DATA AVAILABILITY STATEMENT

Data can be requested from the corresponding author upon reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ethics Committee of West China Hospital (No. 2021-179). All human samples were obtained with informed content.

AUTHORSHIP

JW and JLY designed the outline; JW, MJX, and BHX performed the experiments; JW, QL, and YZL analyzed the data. JW and JLY wrote the manuscript; JW, YZL and JLY supervised and edited the manuscript. All authors read and approved the final manuscript.

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REFERENCES

1. Sahai E, Astsaturov I, Cukierman E, DeNardo DG, Egeblad M, Evans RM, et al. A framework for advancing our understanding of cancer-associated fibroblasts. Nat Rev Cancer. 2020;20(3):174-186.
2. Lavie D, Ben-Shmuel A, Erez N, Scherz-Shouval R. Cancer-associated fibroblasts in the single-cell era. Nat Cancer. 2022;3(7):793-807.
3. Zhu GQ, Tang Z, Huang R, Qu WF, Fang Y, Yang R, et al. CD36(+) cancer-associated fibroblasts provide immunosuppressive microenvironment for hepatocellular carcinoma via secretion of macrophage migration inhibitory factor. Cell Discov. 2023;9(1):25.
4. Kumar V, Ramnarayanan K, Sundar R, Padmanabhan N, Srivastava S, Koiwa M, et al. Single-cell atlas of lineage states, tumor microenvironment, and subtype-specific expression programs in gastric cancer. Cancer Discov. 2022;12(3):670-691.
5. Luo X, Zheng E, Wei L, Zeng H, Qin H, Zhang X, et al. The fatty acid receptor CD36 promotes HCC progression through activating Src/PI3K/AKT axis-dependent aerobic glycolysis. Cell Death Dis. 2021;12(4):328.
6. Wang H, Franco F, Tsui YC, Xie X, Trefny MP, Zappasodi R, et al. CD36-mediated metabolic adaptation supports regulatory T cell survival and function in tumors. Nat Immunol. 2020;21(3):298-308.
7. Ma X, Xiao L, Liu L, Ye L, Su P, Bi E, et al. CD36-mediated ferroptosis dampens intratumoral CD8(+) T cell effector function and impairs their antitumor ability. Cell Metab. 2021;33(5):1001-1012.e5.
8. Yang P, Qin H, Li Y, Xiao A, Zheng E, Zeng H, et al. CD36-mediated metabolic crosstalk between tumor cells and macrophages affects liver metastasis. Nat Commun. 2022;13(1):5782.
9. Pascual G, Avgustinova A, Mejetta S, Martin M, Castellanos A, Attolini CS, et al. Targeting metastasis-initiating cells through the fatty acid receptor CD36. Nature. 2017;541(7635):41-45.
10. Fang Y, Shen ZY, Zhan YZ, Feng XC, Chen KL, Li YS, et al. CD36 inhibits beta-catenin/c-myc-mediated glycolysis through ubiquitination of GPC4 to repress colorectal tumorigenesis. Nat Commun. 2019;10(1):3981.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.