INTRODUCTION

As one of the leading causes of cancer-related deaths, colon cancer is still a major public health issue worldwide.\(^1,2\) Approximately 15%–25% patients were diagnosed with metastasis\(^3\) and 5-year survival rate of metastasis patients is only 10%\(^4\), so there is an urgent need to determine the mechanisms involved.

The recombination signal-binding protein for immunoglobulin kappa J region (RBP-J\(\kappa\)) is a nuclear factor that is required for Notch signalling.\(^5\) RBP-J\(\kappa\) was originally identified from the nuclei of mouse pre-B cells as a DNA-binding protein, and the typical RBP-J\(\kappa\) DNA-binding site contains immunoglobulin J\(\kappa\) recombination signal sequences (5′-CACTGTG-3′).\(^6\) Notch pathway is abnormally activated in human cancer through chromosome rearrangement, activation point mutation or expression pattern change. Therefore, Notch pathway is the target of many kinds of human cancer treatment. The activation of Notch receptor can make \(\gamma\)-Secretase work as hydrolyase and release the intracellular segment of Notch receptor, which can interact with...
RBP-Jκ. By targeting Notch pathway with secretase inhibitor, the production of NICD is prevented and the RBP-Jκ-dependent transcriptional activity is regulated. Recently, RBP-Jκ has been shown to be overexpressed in various human cancers, such as osteosarcoma,7 glioblastoma8 and squamous cell skin carcinoma,9 indicating that it may be an oncogene or act as a tumour promoter. But secretase inhibitor is not like knocking down RBP-Jκ which can inhibit the proliferation of cancer cells to the greatest extent. This shows that the function of promoting tumour cell proliferation of RBP-Jκ is not completely dependent on Notch signal.10 Although a recent study reported that RBP-Jκ knockdown suppressed oral cancer cell EMT,11 the exact function of RBP-Jκ in colon cancer metastasis remains unclear.

Metastasis is a complicated process that not only involves cancer cells but also the tumour microenvironment (TME).12 Monocytes enter tumour lesions and polarize to various subtypes of macrophage as different stimulants in the TME. Macrophage polarize into the M1 type by interferon-gamma and lipopolysaccharide or into the M2 type (also known as TAMs) by IL-4 and IL-13.13 TAMs (tumour-associated macrophages) play a key role in tumour development; however, the function of TAMs in colon cancer metastasis remains unclear.

Therefore, in this study, we explored the clinical significance of the expression of RBP-Jκ and the infiltration of TAMs, and the revealed the underlining mechanisms of RBP-Jκ and TAMs in colon cancer metastasis.

2 MATERIALS AND METHODS

2.1 Bioinformatic analysis

GEIPA (geipa2.cancer-pku.cn/), TGCA (www.cancer.gov) and Oncomine databases (www.oncomine.org) were searched to analyse RBP-Jκ expression in colon cancer. Expression data for RBP-Jκ in colon cancer tissues and normal tissues were extracted and analysed. TIMER (https://cistrome.shinyapps.io/timer/) was used to analyse immune cell infiltration.

2.2 Patients

All protocol was approved by the Ethics Committee of Xi’an Jiaotong University. All patients provided informed consent before surgery. A total of 201 patients with colon adenocarcinoma were enrolled from The First Affiliated Hospital of Xi’an Jiaotong University between January 2011 and January 2013. Each patient was histologically diagnosed with colon adenocarcinoma. All patients did not receive adjuvant chemotherapy or radiotherapy before surgical resection. TNM stage was classified according to the eighth edition of the American Joint Committee on Cancer.14 Patients were regularly followed up until January 2018, and 115 patients died and 86 patients were alive till the last follow-up. Tissue specimens (in paraffin blocks) were retrieved from the Pathology Department and were used for immunohistochemistry (IHC).

2.3 Immunohistochemical staining

Formalin-fixed and paraffin-embedded tissue samples of human and mouse xenograft tissues were used for IHC based on a previous study.15 The antibodies used were shown in Table S1. The stained sections were semiquantitatively scored based on a previous study.16

2.4 Cell culture and transfection

The human colon adenocarcinoma cell lines RKO and SW480 and a human monocyte cell line, Thp-1, were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Tumour cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Hyclon, US) containing 10% (v/v) foetal bovine serum (FBS). Thp-1 cells were cultured in Roswell Park Memorial Institute medium-1640 (RPMI1640, Hyclon, US) containing 10% FBS in a humidified incubator with 5% CO₂ at 37°C. Moreover, colon cancer cells and macrophages were cocultured in Transwell chambers with pore sizes of 0.4 or 8.0 μm (Corning, Corning, NY, USA). A lentiviral vector carrying RBP-Jκ short hairpin RNAs (shRNA; GENECHEM Co. Ltd., Shanghai, China) was used to silence the expression of RBP-Jκ in RKO cells, and a lentiviral vector carrying RBP-Jκ cDNA (GENECHEM Co. Ltd.) was used to overexpress RBP-Jκ in SW480 cells. For cell transfection, cells were seeded and grown to approximately 80% confluency and were then transfected with these lentiviruses according to the manufacturer’s instructions. After 3 days, the transfected cells were further cultured in DMEM containing 4 μg/ml puromycin to establish stable cell sublines; these cells were named RKO-shR (RKO cells with silenced RBP-Jκ expression), RKO-NC (negative control of RKP-shR cells), SW480-R (SW480 cells with RBP-Jκ overexpression) and SW480-NC (negative control of SW480-R cells). Furthermore, tumour-associated macrophages (TAMs) were plated in 6- or 24-well plates for transfection with TGF-β1 small interfering RNA (GenePharma Co. Ltd., Shanghai, China) or a plasmid carrying TGF-β1 cDNA (GenePharma) using Turbo-Fect™ (Thermo Fisher Scientific, Waltham, MA, USA). The RKO-shR cells in 6-well plates were also transfected with a plasmid carrying C-X-C motif chemokine 11 (CXCL11) cDNA (GenePharma) using Turbo-Fect™ (Thermo Fisher Scientific), and the SW480-R cells in 6-well plates were transfected with CXCL11 siRNA (GenePharma) using Turbo-Fect™ (Thermo Fisher Scientific). mRNA expression was determined after 24 h by real-time PCR (qPCR). Total cell protein was extracted for Western blot analysis after 48 h.

2.5 RNA isolation and qPCR

Total RNA was isolated from cells using Fast200 (Tiangen, Beijing, China) and was then reverse transcribed into cDNA using Prime Script™ RT Master Mix (Takara, Shiga, Japan) according to the manufacturer’s protocols. The relative expression of RBP-Jκ, TGF-β1,
CXCL11 and GAPDH mRNA was measured by qPCR with SYBR® Premix Ex Taq™ II (Perfect Real Time, Takara); the specific primer sequences are shown in Table S2. Their relative levels were quantified using the $2^{-\Delta\Delta C_T}$ method against the GAPDH level in each cell line. The experiments were performed in triplicate and were repeated three times.

2.6 | Protein extraction and western blot analysis

Western blot analysis was performed according to a previous study. The antibodies used were shown in Table S1. The experiments were repeated three times.

2.7 | TAMs induction and flow cytometry

To induce TAMs, Thp-1 cells were plated in 6- or 24-well plates at a density of $3 \times 10^6$/ml and were treated with 100 ng/ml phorbol 12-myristate 13-acetate (PMA) (Sigma) in the dark for 24 h and the cells were further treated with 20 ng/ml interleukin 4 (IL-4) (Sigma) and 20 ng/ml IL-13 (Sigma) for 36 h. The expression of CD68 and CD163 was used to identify type 2 TAMs. Next, TAMs were digested with an ethylene diamine tetra acetic acid-free pancreatic enzyme solution and were collected and washed twice with phosphate-buffered saline (PBS), incubated with FcBlock (564219, BD Biosciences, Franklin Lakes, NJ, USA) at room temperature for 10 min and incubated with CD68 monoclonal antibody (11–0689, Thermo Fisher Scientific) and CD163 monoclonal antibody (12–1639, Thermo Fisher Scientific) in the dark for 30 min at 37°C. The cells were then measured using a flow cytometer and analysed with FlowJo software (BD Biosciences).

2.8 | Tumour cell migration and invasion assays

Tumour cell migration and invasion ability were assessed using 24-well Transwell chambers with a pore size of 8 μm (Corning). For the invasion assay, the Transwell filters were precoated with 50 μl of 200 mg/ml Matrigel (356234, BD Biosciences). In brief, $1 \times 10^5$ colon cancer cells (for the migration assay) or $3 \times 10^5$ colon cancer cells (for the invasion assay) in 200 μl of FBS-free medium were plated in the upper chamber, and the serum-supplemented medium or TAMs were used as attractants in the lower chamber. Then, the cells were incubated at 37°C for 24 h, and cells migrating or invading the underside of the filters were fixed in 100% methanol, stained with a 0.5% crystal violet solution and counted under a microscope (Olympus Corporation, Tokyo, Japan). The means of the triplicate assays were used for statistical analysis.

2.9 | Wound healing assay

Cells were seeded in 6-well plates. Then, a wound was created across the entire well using a sterile pipette tip. After washing with PBS, the cells were further cultured in a serum-free medium for an additional 48 h, and then, wound closure was measured after capturing photographic images from six randomly selected microscopic fields. The means of the triplicate assays were used for statistical analysis.

2.10 | Enzyme-linked immune sorbent assay

Cell culture supernatants were harvested, and the concentrations of TGF-β1 and CXCL11 were determined using TGF-β1 and CXCL11 ELISA kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s protocols. The assays were conducted in triplicate and were repeated three times.

2.11 | Animal experiments

The animal experiments in this study were approved by the Institutional Animal Care and Use Committee of Xi'an Jiaotong University. Four-week-old male BALB/c nude mice were purchased from the Animal Center of Xi'an Jiaotong University and housed in a specific pathogen-free facility with free access to autoclaved food and water. The mice were randomized into 12 groups (n = 6) and were implanted subcutaneously in the back with the following: $1 \times 10^6$ RKO-NC, RKO-shR, RKO, RKO mixed with macrophages (1:1), RKO-shR mixed with M2-NC2 cells (1:1), SW480-NC, SW480-R, SW480, SW480 mixed with macrophages (1:1), SW480-R mixed with M2-NC1 (1:1), or SW480-R mixed with M2-siT (1:1) in 100 μl of RPMI1640/Matrigel (356234, BD Biosciences). The mice were monitored for 28 days and were then sacrificed for resection of xenografted tumours and immunohistochemical staining of the tumour sections. Additionally, another set of mice were also randomized into 12 groups (n = 6) for tail vein injections with $1 \times 10^6$ RKO-NC, RKO-shR, RKO, RKO mixed with macrophages (1:1), RKO-shR mixed with M2-NC2 (1:1), SW480-R, SW480 mixed with macrophages (1:1), SW480-R mixed with M2-NC1 (1:1), or SW480-R mixed with M2-siT (1:1) in 100 μl of PBS. The mice were monitored for 8 weeks and were then sacrificed for lung resection and identification of lung metastasis after haematoxylin and eosin staining of the lung sections.

2.12 | RNA sequencing

Total RNA prepared from RKO-NC or RKO-shR cells (see qPCR section) was purified and quantified for construction of RNA sequencing libraries. Then, the libraries were sequenced on the Illumina HiSeq platform; reads containing adapter or poly-N or of low quality were removed, and high-quality and clean reads were further analysed (i.e. they were Q20 [>96%], Q30 [>92%], with an uncertainty rate [<0.01%] for the clean data). The mapped reads were obtained using
Tophat2 after alignment to the reference of the human genome. The number of mapped clean reads for each unigene was counted and then normalized to reads/kb/million reads to calculate the unigene expression levels.

### 2.13 Statistical analysis

The data are expressed as the mean ± standard deviation for each case number. Differences between groups were analysed using Student’s t-test, and differences among multiple variable groups were analysed using one way ANOVA analysis. The associations between the percentage of positive staining and clinical parameters were assessed using Pearson’s χ² test. Moreover, overall survival (OS), which was defined as the time from tumour diagnosis to patient death or the last follow-up, was used to generate Kaplan-Meier curves, was statistically analysed using the log-rank test and was further assessed using univariate and multivariate analyses. All statistical analyses were conducted using SPSS 22.0 software (International Business Machines Corporation, Armonk, NY, USA) with two-side analysis, and a p value < 0.05 was considered statistically significant for levels of *p < 0.05, **p < 0.01 and ***p < 0.001.

### 3 RESULTS

#### 3.1 RBP-Jκ overexpression was associated with macrophage infiltration in colon cancer tissues

GEPIA was used to assess the expression of RBP-Jκ in colon cancer tissues. We found that RBP-Jκ expression in colon cancer tissues (n = 275) was significantly higher than that of normal colon tissue (n = 41, p < 0.01; Figure S1A). Survival analysis showed that high expression of RBP-Jκ indicated shorter OS (p = 0.048) and DFS (p = 0.060; Figure S1B–S1C). Then, we used Oncomine to further confirm this result (p < 0.001; Figure S1D–S1G). TCGA data showed that RBP-Jκ was overexpressed in colon cancer tissues (31 pairs, p = 0.047; 478 tumour tissues vs 31 para-carcinoma tissues, p = 0.046; Figure S1H–S1I). Next, we collected clinical parameters of 247 patients from TCGA data set. Correlation analysis showed that high expression of RBP-Jκ was associated with depth of tumour invasion (p < 0.001) and distance metastasis (p = 0.022, Table S3). The above analysis results implicated that RBP-Jκ was overexpressed in colon cancer tissues and was associated with metastasis.

Finally, we used TIMER to analyse the relevance between immune cell infiltration and RBP-Jκ in colon cancer. Results showed that the macrophage infiltration was associated with overall survival of colon cancer patients. That was patients with high macrophage infiltration had a shorter overall survival than patients with low macrophage infiltration (p = 0.035; Figure S1J). RBP-Jκ was positively correlated with macrophage infiltration (r = 0.486, p = 2.72e-25; Figure S1K). Further analysis revealed that RBP-Jκ was positively correlated with CD163, which is the marker of M2 type macrophage (r = 0.47, p = 1.02 × 10⁻²²; Figure S1L).

#### 3.2 RBP-Jκ and CD163 were independent prognostic predictors of colon cancer patients

To verify the results above, we compared the expression of RBP-Jκ and CD163 in colon cancer and normal tissues from 201 patients (Figure 1A). Our IHC data showed that high RBP-Jκ expression was observed in 124 colon cancer cases (61.69%) compared to 41 cases (20.40%) of para-tumour tissues (p = 0.001; Table 1). High CD163 expression was observed in 103 cases (51.24%) of colon cancer versus 42 cases (20.90%) of para-tumour tissues, thus indicating that TAMs had more infiltration in colon cancer tissues (p < 0.001; Table 1). Moreover, RBP-Jκ expression exhibited a significant positive association with CD163 (r = 0.562, p < 0.001, Table S4).

In addition, high RBP-Jκ expression was associated with dedifferentiation (p = 0.037), invasion beyond the propria muscularis (p = 0.037), lymph node metastasis (p = 0.004), distance metastasis (p < 0.001) and advanced TNM stage in this cohort of patients (p < 0.01; Table 1). High CD163 expression was associated with invasion depth (p = 0.037), lymph node metastasis (p = 0.021), distant metastasis (p = 0.007) and TNM stage of patients (p = 0.003; Table 1). Univariate analysis results showed that high RBP-Jκ expression (p < 0.001), high CD163 expression (p < 0.001) along with poor tumour differentiation (p < 0.001), invasion beyond the propria muscularis (p = 0.032), lymph node metastasis (p < 0.001), distance metastasis (p < 0.001) and advanced TNM stage (p < 0.001) were associated with shorter OS (Table 2). Multivariate analysis results revealed that RBP-Jκ expression (p = 0.021), CD163 expression (p < 0.001), distant metastasis (p < 0.001) and advanced TNM stage (p < 0.001) were independent prognostic predictors for shorter OS (Table 2).

Moreover, patients with high tumour RBP-Jκ expression had significantly shorter OS (p < 0.001; Figure 1B) and patients with high CD163 expression in colon cancer had a significantly shorter OS (p < 0.001; Figure 1C). Furthermore, combining RBP-Jκ expression and CD163 expression, stratification analysis showed that patients with high RBP-Jκ expression and high CD163 expression had the shortest OS (p < 0.001, Figure 1D, 1E).

#### 3.3 RBP-Jκ promoted colon cancer cell metastasis

Our ex vivo data revealed that RBP-Jκ expression was associated with colon cancer metastasis. Therefore, we established stable sublines of RKO-shR, RKO-NC, SW480-R and SW480-NC cells (Figure 2A, 2B, Figure S4A). Then, we found that RBP-Jκ knockdown significantly reduced tumour cell migration (59.67±8.18 cells per microscopic field in RKO-shR vs. 98.00 ± 6.53 in RKO-NC,
that RBP-Jκ overexpression markedly induced tumour cell migration in SW480-R vs. SW480-NC (98.33 ± 17.56 vs. 56.00 ± 16.82, p = 0.039; Figure 2D). Moreover, tumour cell invasion ability was similar for RKO-shR vs. RKO-NC (26.67 ± 6.24 vs. 53.33 ± 11.47, p = 0.045; Figure 2C) and for SW480-R vs. SW480-NC (85.67 ± 15.04 vs. 35.33 ± 9.61, p = 0.008; Figure 2D). In addition, the wound healing assay showed that RKO-shR had a significantly lower cell migration rate than RKO-NC (27.74% ± 7.85% vs. 59.49% ± 13.44%, p = 0.045; Figure 2E), whereas RBP-Jκ overexpression dramatically induced tumour cell migration for SW480-R vs. SW480-NC (51.54% ± 13.50% vs. 25.05% ± 6.00%, p = 0.036; Figure 2F). Because EMT contributes to increased tumour cell migration and invasion, we assessed the expression of EMT-related proteins (e.g. E-cadherin, N-cadherin, Vimentin and Snail) in these cell sublines. E-cadherin expression was downregulated but N-cadherin, Vimentin and Snail expression was upregulated in RKO-shR compared to RKO-NC cells, whereas the expression pattern was reverse in SW480-R vs. SW480-NC cells (Figure 2G, Figure S4B).

We next evaluated the effect of RBP-Jκ on cell EMT in vivo. First, we established a xenograft tumour model (Figure 3A) in nude mice using RKO-shR, RKO-NC, SW480-R and SW480-NC cells. Then, we assessed the expression of E-cadherin and N-cadherin in xenograft tumour tissue sections and found that the expression of E-cadherin was lower in RKO-NC and SW480-R than that in RKO-shR and SW480-NC, while the expression of N-cadherin was higher in RKO-NC and SW480-R than that in RKO-shR and SW480-NC (Figure 3B, 3C). Moreover, we established lung metastasis model (Figure 3D) in nude mice using RKO-shR, RKO-NC, SW480-R and SW480-NC cells to evaluated the effect of RBP-Jκ on metastasis in vivo. We found that RKO-shR cells formed smaller and fewer metastasis nodules in the lungs than RKO-NC cells, whereas SW480-R cells

![Image](image_url)
formed larger and more metastasis nodules than SW480-NC cells (Figure 3E). Taken together, these data indicate that RBP-Jκ expression promotes colon cancer cell metastasis both in vitro and in vivo.

### 3.4 | TAMs promoted colon cancer cell metastasis by secreting TGF-β1

As high CD163 expression was associated with cancer metastasis, we assessed the effects of CD68⁺CD163⁺TAMs on colon cancer cells. We induced Thp-1 into TAMs and the CD68 and CD163 double-positive rate of TAMs was 87.85% ± 7.19% (Figure S2A). Next, we conducted cell migration, invasion and wound healing assays in RKO and SW480 cells cocultured with TAMs (Figure S2B). The numbers of cells that migrated (65.67 ± 7.51 vs. 89.67 ± 10.02 in RKO; p = 0.029; 43.33 ± 9.61 vs. 82.33 ± 12.01 in SW480; p = 0.012) and invaded (58.00 ± 8.00 vs. 98.33 ± 11.06 in RKO; p = 0.007; 46.67 ± 9.07 vs. 85.33 ± 14.01 in SW480; p = 0.016) increased after coculturing with TAMs (Figure S2C). The wound healing assay showed similar data (53.31% ± 8.17% vs. 79.46% ± 7.19% for RKO; p = 0.007; 46.67 ± 9.07 vs. 85.33 ± 11.06 for SW480; p = 0.016) after coculturing with TAMs (Figure S2D). Meanwhile, Western blot analysis results showed that TAMs were able to induce RKO and SW480 cells to undergo EMT (Figures S2E, S4M).

Furthermore, we found that TGF-β1 expression gradually increased during the TAMs induction process (Figure 4A, Figure S4C). Thus, we established TGF-β1-knockdown TAMs (M2-siT) or negative control cells (M2-NC1) and TGF-β1-overexpressed TAMs (M2-T) or negative control cells (M2-NC2; Figure 4B, 4C, Figure S4D). We then cocultured RKO and SW480 cells with M2-siT or M2-T for use in cell migration, invasion and wound healing assays. The numbers of cells that migrated (63.00 ± 7.55 vs. 39.00 ± 10.15 for RKO; p = 0.030; 56.00 ± 9.54 vs. 35.33 ± 5.69 for SW480; p = 0.032; Figure 4D)

### Table 1: Association of RBP-Jκ and CD163 expression with clinicopathological features from 201 colon cancer patients

| Characteristics          |          |          |          |          |          |          |          |          |
|--------------------------|----------|----------|----------|----------|----------|----------|----------|----------|
|                          | n        | RBP-Jκ High | Low | p-value | CD163 High | Low | p-value |
| Histology Type           |          |           |         |         |          |         |         |         |
| Tumour tissue            | 201      | 124 (61.69%) | 77 (38.31%) | <0.001 | 103 (51.24%) | 98 (48.76%) | <0.001 |
| Para-tumour tissue       | 201      | 41 (20.40%) | 160 (79.60%) |         | 42 (20.90%) | 159 (79.10%) |         |
| Gender                   |          |           |         |         |          |         |         |         |
| Male                     | 125      | 82        | 43      | 0.14    | 70       | 55      | 0.084    |
| Female                   | 76       | 42        | 34      |         | 33       | 43      |         |
| Age                      |          |           |         |         |          |         |         |         |
| <60                       | 91       | 59        | 32      | 0.40    | 50       | 41      | 0.34     |
| ≥60                       | 110      | 65        | 45      |         | 53       | 57      |         |
| Tumour location          |          |           |         |         |          |         |         |         |
| Right colon              | 113      | 65        | 48      | 0.16    | 53       | 60      | 0.16     |
| Left colon               | 88       | 59        | 29      |         | 50       | 38      |         |
| Differentiation          |          |           |         |         |          |         |         |         |
| Well/Moderate            | 169      | 99        | 70      | 0.037   | 82       | 87      | 0.076    |
| Poor                     | 32       | 25        | 7       |         | 21       | 11      |         |
| Depth of invasion        |          |           |         |         |          |         |         |         |
| Tis/T1/T2                | 18       | 7         | 11      | 0.037   | 5        | 13      | 0.037    |
| T3/T4                    | 183      | 117       | 66      |         | 98       | 85      |         |
| Lymph node metastasis    |          |           |         |         |          |         |         |         |
| No                       | 121      | 65        | 56      | 0.004   | 54       | 67      | 0.021    |
| Yes                      | 80       | 59        | 21      |         | 49       | 31      |         |
| Distant metastasis       |          |           |         |         |          |         |         |         |
| No                       | 173      | 97        | 76      | <0.001  | 82       | 91      | 0.007    |
| Yes                      | 28       | 27        | 1       |         | 21       | 7       |         |
| TNM Stage                |          |           |         |         |          |         |         |         |
| I/II                     | 112      | 56        | 56      | <0.001  | 47       | 65      | 0.003    |
| III/IV                   | 89       | 68        | 21      |         | 56       | 33      |         |
and invaded (49.67 ± 10.02 vs. 24.67 ± 5.51 for RKO; p = 0.019; 49.00 ± 9.17 vs. 32.33 ± 3.06 for SW480; p = 0.040; Figure 4D) and the cell migration rate (65.02% ± 8.31% vs. 34.50% ± 5.60% for RKO; p = 0.006; 56.32% ± 10.94% vs. 28.84% ± 7.48% for SW480; p = 0.023; Figure 4E) all decreased after coculturing with M2-siT. In contrast, the numbers of tumour cells that migrated (66.00 ± 13.53 vs. 99.00 ± 10.15 for RKO; p = 0.023; Figure 4E) and invaded (52.00 ± 7.55 vs. 86.67 ± 8.02 for RKO; p = 0.006; 50.67 ± 6.51 vs. 92.67 ± 10.50 for SW480; p = 0.004; Figure 4D) and the cell migration rate (44.61% ± 7.59% vs. 75.19% ± 12.48% for RKO; p = 0.022; 44.55% ± 5.65% vs. 66.37% ± 6.19% for SW480; p = 0.011; Figure 4E) were induced after coculturing with M2-T. Moreover, our Western blot analysis results also showed that knockdown of TGF-β1 expression in TAMs reversed the effect of TAMs on tumour cell EMT and that TGF-β1 overexpression in TAMs facilitated this effect (Figure 4F, Figure S4E). In addition, TAMs activated the TGF-β1/Smad3 pathway in both RKO and SW480 cells (Figure 4G, Figure S4F).

In addition, we also assessed the effect of TAMs on the induction of colon cancer cell EMT in the nude mouse xenograft tumour (Figure 5A) by using RKO or SW480 cells with or without macrophages. CD163 immunostaining of the mesenchyme in the cocultured group showed higher expression than that of the RKO or SW480-alone groups, whereas E-cadherin immunostaining in the cocultured group was lower than the RKO or SW480-alone groups. The expression of N-cadherin was higher in the cocultured group than that in the RKO or SW480-alone groups (Figure 5B, 5C). Lung metastasis models (Figure 5D) were established by using RKO or SW480 cells with or without macrophages. Similarly, the lung metastasis model provided comparable data, that is RKO cells or SW480

| Characteristics | n   | Univariate analysis | Multivariate analysis |
|-----------------|-----|---------------------|----------------------|
|                 |     | HR (95% CI)         | p-value              |
|                 |     |                     |                      |
| Gender          |     |                     |                      |
| Male            | 125 | 1                    | 0.21                 |
| Female          | 76  | 0.782 (0.531–1.151)  |                      |
| Age             |     |                     |                      |
| <60             | 91  | 1                    | 0.46                 |
| ≥60             | 110 | 0.871 (0.604–1.256)  |                      |
| Tumour location |     |                     |                      |
| Right colon     | 113 | 1                    | 0.82                 |
| Left colon      | 88  | 1.043 (0.721–1.508)  |                      |
| Differentiation |     |                     |                      |
| Well/Moderate   | 169 | 1                    | <0.001               |
| Poor            | 32  | 2.198 (1.424–3.392)  |                      |
| Depth of invasion|   |                     |                      |
| Tis/T1/T2       | 18  | 1                    | 0.032                |
| T3/T4           | 183 | 2.667 (1.088–6.538)  |                      |
| Lymph node metastasis | |                     |                      |
| No              | 121 | 1                    | <0.001               |
| Yes             | 80  | 3.174 (2.182–4.617)  |                      |
| Distant metastasis |   |                     |                      |
| No              | 173 | 1                    | <0.001               |
| Yes             | 28  | 6.047 (3.783–9.665)  | 1.522–4.238          |
| TNM Stage       |     |                     |                      |
| I/II            | 112 | 1                    | <0.001               |
| III/IV          | 89  | 4.105 (2.776–6.070)  | 2.964 (1.924–4.566)  |
| RBP-κ           |     |                     |                      |
| High            | 124 | 1                    | <0.001               |
| Low             | 77  | 0.233 (0.147–0.370)  | 0.542 (0.322–0.912)  |
| CD163           |     |                     |                      |
| High            | 103 | 1                    | <0.001               |
| Low             | 98  | 0.270 (0.180–0.406)  | 0.421 (0.269–0.657)  |

| Characteristics | n   | Univariate analysis | Multivariate analysis |
|-----------------|-----|---------------------|----------------------|
|                 |     |                     |                      |
|                 |     | HR (95% CI)         | p-value              |
|                 |     |                     |                      |
| Gender          |     |                     |                      |
| Male            | 125 | 1                    | 0.21                 |
| Female          | 76  | 0.782 (0.531–1.151)  |                      |
| Age             |     |                     |                      |
| <60             | 91  | 1                    | 0.46                 |
| ≥60             | 110 | 0.871 (0.604–1.256)  |                      |
| Tumour location |     |                     |                      |
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| Differentiation |     |                     |                      |
| Well/Moderate   | 169 | 1                    | <0.001               |
| Poor            | 32  | 2.198 (1.424–3.392)  |                      |
| Depth of invasion|   |                     |                      |
| Tis/T1/T2       | 18  | 1                    | 0.032                |
| T3/T4           | 183 | 2.667 (1.088–6.538)  |                      |
| Lymph node metastasis | |                     |                      |
| No              | 121 | 1                    | <0.001               |
| Yes             | 80  | 3.174 (2.182–4.617)  |                      |
| Distant metastasis |   |                     |                      |
| No              | 173 | 1                    | <0.001               |
| Yes             | 28  | 6.047 (3.783–9.665)  | 1.522–4.238          |
| TNM Stage       |     |                     |                      |
| I/II            | 112 | 1                    | <0.001               |
| III/IV          | 89  | 4.105 (2.776–6.070)  | 2.964 (1.924–4.566)  |
| RBP-κ           |     |                     |                      |
| High            | 124 | 1                    | <0.001               |
| Low             | 77  | 0.233 (0.147–0.370)  | 0.542 (0.322–0.912)  |
| CD163           |     |                     |                      |
| High            | 103 | 1                    | <0.001               |
| Low             | 98  | 0.270 (0.180–0.406)  | 0.421 (0.269–0.657)  |
cells formed smaller and fewer metastasis nodules in the lungs than that with macrophages (Figure 5E).

3.5 | RBP-Jκ promotes colon cancer cell metastasis through inducing TAMs secret TGF-β1

Taken together, our data show that RBP-Jκ and TAMs participate in promoting colon cancer metastasis. We then detected the relationship between colon cancer expression RBP-Jκ and TAMs and found that TGF-β1 expression was downregulated or upregulated in TAMs when cocultured with RKO-shR or SW480-R cells (Figure 6A, Figure S4G). We further evaluated whether RBP-Jκ induced colon cancer cell migration and invasion through TGF-β1 expression in TAMs by establishing six different coculture systems: i) RKO-NC coculture with M2-NC2; ii) RKO-shR coculture with M2-NC2; iii) RKO-shR coculture with M2-T; iv) SW480-NC coculture with M2-NC1; v) SW480-R coculture with M2-NC1; and vi) SW480-R coculture with M2-siT, for migration, invasion and wound healing assays. Our data showed that downregulation or upregulation of RBP-Jκ expression in colon cancer cells suppressed or promoted tumour cell migration and invasion capacities, while upregulation or downregulation of TGF-β1 expression in TAMs reversed the downregulation or upregulation effect of RBP-Jκ on colon cancer cells (Figure 6B, 6C). Furthermore, our Western blot analysis results revealed that downregulation or upregulation of RBP-Jκ expression in colon cancer cells suppressed or promoted tumour cell migration and invasion capacities, while upregulation or downregulation of TGF-β1 expression in TAMs reversed the downregulation or upregulation effect of RBP-Jκ on colon cancer cells (Figure 6D, Figure S4H).
FIGURE 4 TAMs promote colon cancer cell metastasis through TGF-β1 in vitro. (A) Western blot analysis. M2 type TAMs expressed more TGF-β1 vs. M1 or monocytes. (B) Western blot analysis. TGF-β1 was silenced and overexpressed in M2 type TAMs after transfection of TGF-β1 siRNA and cDNA, respectively. (C) Real-time PCR analysis. TGF-β1 mRNA was silenced and overexpressed in M2 type TAMs after transfection of TGF-β1 siRNA and cDNA, respectively. (D) Transwell migration and invasion assays. Silencing or overexpression of TGF-β1 in TAMs impaired or enhanced the ability of RKO and SW480 cell migration and invasion, respectively. (E) Wound healing assay. Silencing or overexpression of TGF-β1 in TAMs impaired or enhanced the ability of RKO and SW480 cells to migrate, respectively. (F) Western blot analysis. Silencing and overexpression of TGF-β1 in TAMs impaired and enhanced EMT of RKO cells and SW480 cell, respectively. (G) Western blot analysis. Silencing or overexpression of TGF-β1 in TAMs suppressed or activated the TGF-β1/Smad3 pathway in RKO and SW480 cells, respectively.
Additionally, we then established a xenograft tumour model with RKO-shR plus M2-NC2 or M2-T or SW480-R plus M2-NC1 or M2-siT (Figure 7A). All the tumour tissues in these four groups expressed CD163 in the mesenchyme, whereas tumours in the RKO-shR groups expressed low levels of RBP-Jκ and tumours in SW480-R groups exhibited high levels of RBP-Jκ. E-cadherin expression in the RKO-shR plus M2-T group or SW480-R plus M2-siT groups, while the expression of N-cadherin showed the opposite phenomenon (Figure 7B, 7C). Similarly, from the lung metastasis model (Figure 7D) also confirmed these findings, which showed that metastasis nodules in the RKO-shR plus M2-T or SW480-R plus M2-NC1 groups were larger and more prevalent than those in the RKO-shR plus M2-NC2 or M2-siT groups (Figure 7E). These data suggest that RBP-Jκ expression in colon cancer cells promotes tumour cell metastasis via TAM expression of TGF-β1.

3.6 Colon cancer cell with overexpressed RBP-Jκ induced TAMs to express TGF-β1 by secretion of CXCL11

We demonstrated that colon cancer cells with RBP-Jκ overexpression induced TAMs to express TGF-β1, but the underlying molecular events still remained to be determined. Therefore, we performed an RNA sequencing assay to identify the molecules that were possibly involved. Among the differentially expressed RNAs, there were 57 genes downregulated (Figure 8A, 8B). And among the 57 genes, there were 7 secretory proteins which were associated with immune and inflammation (CP, ceruloplasmin; A2 M, alpha-2-macroglobulin; CXCL11, C-X-C motif chemokine ligand 11; IGFL2, IGF like family member 2; FGB, fibrinogen beta chain; FGA, fibrinogen alpha chain; and IGFBP1, insulin-like growth factor binding protein 1). Then, we used TIMER to analyse the relevance between macrophage infiltration and the 7 genes. Results showed that CP, A2 M, CXCL11 and IGFL2 were positively related to macrophage infiltration, while FGB and FGA were negatively related to macrophage infiltration, and IGFBP1 had little to do with macrophage infiltration (Figure 8C). Next, we treated TAMs separately with CP, A2 M, CXCL11 and IGFL2 and found that CXCL11 induced TAMs express TGF-β1 (Figure 8D, Figure S4I) and the concentration of TGF-β1 in the TAMs-cultured supernatant after CXCL11 treatment was significantly induced (765.24 ± 82.77 vs. 2286.74 ± 241.23 pg/mL; p < 0.001; Figure 8E). The sequencing analysis was confirmed by real-time PCR and Western blot analyses which showed that the CXCL11 mRNA and protein levels were lower in RKO-shR and SW480-NC cells than those in RKO-NC and SW480-R cells, respectively (Figure 8F, 8G, Figure S4J). Moreover, the RKO-shR or SW480-NC-cultured supernatant concentration of CXCL11 was also significantly lower than that of RKO-NC or SW480-R (918.15 ± 158.34 vs. 257.94 ± 57.86 pg/mL; p = 0.002;
We further demonstrated that RBP-Jκ facilitated TAMs to express TGF-β1 via CXCL11 secretion after overexpression of CXCL11 in RKO-shR cells (RKO-shR-C) versus RKO-shR-NC cells as a control or after knockdown of CXCL11 expression in SW480-R cells (SW480-R-siC) vs. SW480-R-NC cell as a control (Figure 8I, 8K, Figure S4K). We cocultured TAMs with RKO-NC, RKO-shR-NC, RKO-shR-C, SW480-R, SW480-R-NC or SW480-R-siC cells for Western blot analysis of TGF-β1. We found that RKO-shR-C cells or SW480-R and SW480-R-NC cells stimulated TAMs to express TGF-β1 (Figure 8K, Figure S4L). These data indicate that RBP-Jκ induces TAMs to express TGF-β1 by increasing colon cancer cell secretion of CXCL11.

FIGURE 6 RBP-Jκ promotes colon cancer cell metastasis through the induction of TGF-β1 expression in TAMs in vitro. (A) Western blot analysis. Silencing or overexpression of RBP-Jκ suppressed or enhanced M2 type TAMs expression of TGF-β1, respectively. (B) Transwell migration and invasion assays. TGF-β1 overexpression in TAMs reversed the effect of RBP-Jκ silencing and resulted in the promotion of RKO-shR cell migration and invasion. Silencing of TGF-β1 in TAMs reversed the effect of RBP-Jκ overexpression and resulted in the inhibition of SW480-R cell migration and invasion. (C) Wound healing assay. TGF-β1 overexpression in TAMs reversed the effect of RBP-Jκ silencing and resulted in the promotion of RKO-shR cell migration. Silencing of TGF-β1 in TAMs reversed the effect of RBP-Jκ overexpression and resulted in the inhibition of SW480-R cell migration. (D) Western blot analysis. TGF-β1 overexpression in TAMs reversed the effect of RBP-Jκ silencing and resulted in the promotion of RKO-shR cell EMT, whereas silencing of TGF-β1 expression in TAMs reversed the effect of RBP-Jκ overexpression and resulted in the inhibition of SW480-R cell EMT.
Although Notch signalling is closely associated with carcinogenesis and the development of colon cancer,18 studies of the effect of RBP-Jκ, the main transcription factor of Notch pathway,5 on colon cancer metastasis remain scarce. Hence, in this study, we first analysed RBP-Jκ expression in colon cancer tissues with TCGA data and found that RBP-Jκ was overexpressed in colon cancer tissues and associated with distant metastasis, poor OS and macrophage infiltration. To strengthen our bioinformation findings, we tested RBP-Jκ expression and TAMs infiltration in human colon cancer tissues and analysed the associations between TAMs infiltration and RBP-Jκ expression, and the characteristics and outcomes of colon cancer patients. We consistently found that high RBP-Jκ expression was related to more infiltration of TAMs in colon cancer tissues. Additionally, high RBP-Jκ and more infiltration of TAMs in colon cancer tissues were associated with metastasis and poor prognosis. Our current data confirmed that RBP-Jκ is closely associated with colon cancer progression; however, these results need to be validated using a larger cohort of patients.

Different macrophage populations possess and express different molecular markers: M1 type play anti-inflammation and antitumour roles19 and are characterized by the high expression of CD80 and CD86,21 whereas M2 type play proinflammation and protumour roles22-24 and express high levels of CD163,25 CD20426 and CD20627 and play as markers of TAMs. In our study, we cocultured CD68+CD163+TAMs with colon cancer cells with different RBP-Jκ expression. We found that colon cancer cells with high RBP-Jκ expression were capable of stimulating TAMs to secrete more TGF-β1, and TAMs further facilitated EMT, migration and invasion of colon cancer cells. Hence, one or more cytokines or growth factors may mediate the signalling from RBP-Jκ in colon cancer cells to TAMs. Using an RNA sequencing assay, we found that RBP-Jκ was able to upregulate the expression of CXCL11 in colon cancer cells. CXCL11 is a member of the CXC chemokine family.28 CXCL11 is the ligand with the highest affinity to CXCR3, followed by CXCL9 and CXCL10.29 As an ELR (Glu-Leu-Arg)-negative CXC chemokine, CXCL11 can generally attenuate angiogenesis and thus have an antitumour effect.30 In this study, we established a xenograft tumour model for tail vein injections with RKO-shR plus M2-NC2(1:1) or M2-T (1:1)or SW480-R plus M2-NC1(1:1) or M2-siT (1:1), the number of cells and injectate
volume were determined by our pre-experiment according to previous studies. However, there may be some possible limitations in this method. Although the method of establishing metastatic lung cancer model by injecting cancer cells into mouse tail vein is often used, there are many differences in the evaluation indexes of this kind of lung cancer model, and there is no systematic discussion on the window period suitable for the experiment and the repeatability of the model. Therefore, further efforts are needed to improve our metastasis model in our future research.

To our knowledge, our study being conducted at present is the first one that investigates the association between RBP-Jκ and CXCL11 expression in colon cancer, as well as high RBP-Jκ and
CXCL11 co-expression in relation to malignant colon cancer transformation. Interestingly, in our research, we found that in colon cancer cells, RBP-Jκ-induced CXCL11 was able to enhance the function of TAMs. At this point, we found a loop of colon cancer cells and TAMs in which colon cancer cells and TAMs promoted the functions of each other and thus led to colon cancer metastasis (Figure S3).

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CONFLICT OF INTEREST STATEMENT
All the authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS
Mengjie Liu: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Writing-original draft (equal). Xiao Fu: Data curation (equal); Formal analysis (equal). Lili Jiang: Data curation (supporting); Formal analysis (supporting). Jiequn Ma: Funding acquisition (equal); Investigation (supporting). Xiaqiang Zheng: Investigation (supporting). Shuhong Wang: Software (equal); Hui Guo: Supervision (equal); Writing-review & editing (equal). Tao Tian: Writing-review & editing (supporting). Kejun Nan: Supervision (equal); Wenjuan Wang: Funding acquisition (equal); Supervision (equal); Writing-review & editing (equal).

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are openly available at geopia2.cancer-pku.cn, www.cancer.gov, www.oncomine.org, https://cistrome.shinyapps.io/timer/.

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REFERENCES
1. Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65(2):87-108.
2. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. CA Cancer J Clin. 2016;66(2):115-132.
3. Liu J, Zhan Y, Wang JF, et al. Long noncoding RNA LINC01578 drives colon cancer metastasis through a positive feedback loop with the NF-κB/YY1 axis. Mol Oncol. 2020;14:3211-3233.

FIGURE 8 RBP-Jκ facilitates TGF-β1 expression of TAMs by secreting CXCL11. (A) Heat map of differentially expressed genes between RKO-NC and RKO-shR cells. (B) Volcano map of differentially expressed genes between RKO-NC and RKO-shR cells. (C) TIMER analysis. CP, A2 M, CXCL11 and IGFL2 were positively associated with macrophage infiltration. FGA and FGB were negatively associated with macrophage infiltration. IGFBP1 was unrelated to macrophage infiltration. (D) Western blot analysis. CXCL11 upregulated TGF-β1 expression of M2 type TAMs. (E) ELISA. TGF-β1 expression in TAMs was upregulated after CXCL11 treatment. (F) Western blot analysis. Silencing or overexpression of RBP-Jκ downregulated or upregulated the expression of the CXCL11 protein, respectively. (G) Real-time PCR analysis. Silencing or overexpression of RBP-Jκ downregulated or upregulated the expression of CXCL11 mRNA, respectively. (H) ELISA. The CXCL11 concentrations in the cell culture supernatants showed that silencing or overexpression of RBP-Jκ decreased or increased CXCL11 levels, respectively. (I) Western blot analysis. The CXCL11 protein was overexpressed or silenced in RKO-shR and SW480-R cells, respectively, after transfection of CXCL11 cDNA or siRNA. (J) Real-time PCR analysis. CXCL11 mRNA was overexpressed or silenced in RKO-shR and SW480-R cells, respectively, after transfection of CXCL11 cDNA or siRNA. (K) Western blot analysis. TGF-β1 expression in TAMs was up- or downregulated after coculturing with RKO-shR-C or SW480-R-siC cells, respectively. *p < 0.01 and **p < 0.001. NC, negative control.

4. Hermann B, Matthias K, Christian P. Colorectal cancer. Lancet. 2014;383(9927):1490-1502.
5. Tabaja N, Yuan Z, Oswald F, et al. Structure-function analysis of RBP-J interacting and tubulin-associated (RITA) reveals regions critical for repression of Notch target genes. J Biol Chem. 2017;292:10549-10563.
6. Kamaguchi Y, Matsunami N, Yamamoto Y, et al. Purification and characterization of a protein that binds to the recombination signal sequence of the immunoglobulin J kappa segment. Nucleic Acids Res. 1989;17:9015-9026.
7. Gao X, Han D, Fan W. Down-regulation of RBP-J mediates by microRNA-133a suppresses dendritic cells and functions as a potential tumor suppressor in osteosarcoma. Exp Cell Res. 2016;349:264-272.
8. Maciacyzk D, Picard D, Zhao L, et al. CBFA1 is clinically prognostic and serves as a target to block cellular invasion and chemoresistance of EMT-like glioblastoma cells. Br J Cancer. 2017;117:102-112.
9. Al Labban D, Jo SH, Ostano P, et al. Notch-effector CSL promotes squamous cell carcinoma by repressing histone dimethylase KDM6B. J Clin Investig. 2018;128:2581-2599.
10. Yong T, Sun A, Henry MD, et al. Down regulation of CSL activity inhibits cell proliferation in prostate and breast cancer cells. J Cell Biochem. 2011;112(9):2340-2351.
11. Li JY, Huang WX, Zhou X, et al. Numb inhibits epithelialmesenchymal transition via RBP-Jκappa-dependent Notch1/PTEN/FAK signaling pathway in tongue cancer. BMC Cancer. 2019;19:391.
12. Hui L, Chen Y. Tumor microenvironment: Sanctuary of the devil. Cancer Lett. 2015;368:7-13.
13. Ramanathan S, Jagannathan N. Tumor associated macrophage: A review on the phenotypes, traits and functions. Iran J Cancer Prev. 2014;7(7):1-8.
14. Amin MB, Greene FL, Edge SB, et al. The eighth edition AJCC cancer staging manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. CA Cancer J Clin. 2017;67:93-99.
15. Liu M, Jiang L, Fu X, et al. Cytoplasmic liver kinase B1 promotes the growth of human lung adenocarcinoma by enhancing autophagy. Cancer Sci. 2018;109:3055-3067.
16. Wang WJ, Yao Y, Jiang LL, et al. Increased LEF1 expression and decreased Notch2 expression are strong predictors of poor outcomes in colorectal cancer patients. Dis Markers. 2013;35(5):395-405.
17. Ding JX, Jin W, Chen CM, et al. Tumor associated macrophage × cancer cell hybrids may acquire cancer stem cell properties in breast cancer. PLoS One. 2012;7(7):e41942.
18. Miyamoto S, Nakanishi M, Rosenberg DW. Suppression of colon carcinogenesis by targeting Notch signaling. Carcinogenesis. 2013;34:2415-2423.
19. Sawa-Wejksza K, Kandefer-Szerszen M. Tumor-associated macrophages as target for antitumor therapy. Arch Immunol Ther Exp. 2018;66:97-111.
20. Inoue Y, Fukui H, Xu X, et al. Colonic M1 macrophage is associated with the prolongation of gastrointestinal motility and obesity in mice treated with vancomycin. Mol Med Rep. 2019;19:2591-2598.

21. Chen X, Yang B, Tian J, et al. Dental follicle stem cells ameliorate lipopolysaccharide-induced inflammation by secreting TGF-beta3 and TSP-1 to elicit macrophage M2 polarization. Cell Physiol Biochem. 2018;51:2290-2308.

22. Kodaira H, Koma YI, Hosono M, et al. ANXA10 induction by interaction with tumor-associated macrophages promotes the growth of esophageal squamous cell carcinoma. Pathol Int. 2019;69:135-147.

23. Singhal S, Stadanlick J. Human tumor-associated macrophages/macularfites and their regulation of T cell responses in early-stage lung cancer. Sci Transl Med. 2019;11(479):eaat1500.

24. Zhu C, Kros JM, Cheng C, et al. The contribution of tumor-associated macrophages in glioma neo-angiogenesis and implications for anti-angiogenic strategies. Neuro-oncology. 2017;19:1435-1446.

25. Yan Y, Zhang J, Li JH, et al. High tumor-associated macrophages infiltration is associated with poor prognosis and may contribute to the phenomenon of epithelial-mesenchymal transition in gastric cancer. Onco Targets Ther. 2016;9:3975-3983.

26. Shigeoka M, Urakawa N, Nakamura T, et al. Tumor associated macrophage expressing CD204 is associated with tumor aggressiveness of esophageal squamous cell carcinoma. Cancer Sci. 2013;104:1112-1119.

27. Wang F, Li B, Wei Y, et al. Tumor-derived exosomes induce PD1(+) macrophage population in human gastric cancer that promotes disease progression. Oncogenesis. 2018;7:41.

28. Chalin A, Lefevre B, Devisse C, et al. Circulating levels of CXCL11 and CXCL12 are biomarkers of cirrhosis in patients with chronic hepatitis C infection. Cytokine. 2019;117:72-78.

29. Tokunaga R, Zhang W, Naseem M, et al. CXCL9, CXCL10, CXCL11/ CXCR3 axis for immune activation - A target for novel cancer therapy. Cancer Treat Rev. 2018;63:40-47.

30. Strieter RM, Polverini PJ, Kunkel SL, et al. The functional role of the ELR motif in CXC chemokine-mediated angiogenesis. J Biol Chem. 1995;270:27348-27357.

31. Wculek SK, Malanchi I. Neutrophils support lung colonization of metastasis-initiating breast cancer cells. Nature. 2015;528(7582):413-417.

32. Chiou J, Chang YC, Tsai HF, et al. Follistatin-like protein 1 inhibits lung cancer metastasis by preventing proteolytic activation of osteopontin. Cancer Reserach. 2019;79(24):6113-6125.

SUPPORTING INFORMATION
Additional supporting information may be found in the online version of the article at the publisher’s website.

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