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ORIGINAL RESEARCH

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Platelet-derived microparticles adoptively transfer integrin β3 to promote antitumor effect of tumor-infiltrating T cells

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ABSTRACT

Approximately two-thirds of hepatocellular carcinoma (HCC) is considered a "cold tumor" characterized by few tumor-infiltrating T cells and an abundance of immunosuppressive cells. Cilengitide, an integrin αvβ3 inhibitor, has failed in clinical trials as a potential anticancer drug. This failure implies that integrin αvβ3 may play an important role in immune cells. However, the expression and potential role of integrin αvβ3 in T cells of HCC patients remain unknown. Here, we established two HCC models and found that cilengitide had a dual effect on the HCC microenvironment by exerting both antitumor effect and immunosuppressive effect on T cells. This may partly explain the failure of cilengitide in clinical trials. In clinical specimens, HCC-infiltrating T cells exhibited deficient expression and activation of integrin β3, which was associated with poor T-cell infiltration into tumors. Additionally, integrin β3 functioned as a positive immunomodulatory molecule to facilitate T-cell infiltration and T helper 1-type immune response in vitro. Furthermore, T cells and platelet-derived microparticles (PMPs) co-culture assay revealed that PMPs adoptively transferred integrin β3 to T cells and positively regulated T cell immune response. This process was mediated by clathrin-dependent endocytosis and macropinocytosis. Our data demonstrate that integrin β3 deficiency on HCC-infiltrating T cells may be involved in shaping the immunosuppressive tumor microenvironment. PMPs transfer integrin β3 to T cells and positively regulate T cell immune response, which may provide a new insight into immune therapy of HCC.

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KEYWORDS
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Introduction

Hepatocellular carcinoma (HCC) is the most prevalent liver malignancy and ranks as the fourth leading cause of cancer-related mortality.[1] Approximately 63% of HCC is referred to as a "cold tumor" characterized by few tumor-infiltrating lymphocytes (TILs).[2] HCC patients with high densities of $CD3^+$ and $CD8^+$ TILs in the tumor have longer overall survival.[3] The presence of effector T cells and a high intertumoral $CD8^+$ T cells density are favorable biomarkers for responsiveness to immunotherapy.[4] Thus, it is imperative to comprehend how T cells are attracted to the tumor microenvironment (TME). HCC typically arises from liver fibrosis characterized by excessive deposition of extracellular matrix (ECM) proteins in the TME. Environmental factors, such as aberrant vasculature, chemokines,[5] and ECM components,[6] influence the efficacy and manner of T-cell infiltration into tumors. Emerging evidence suggests that increased expression of integrins on T cells is sufficient to facilitate chemokine-independent migration.[7] T cells may adhere to and crawl along ECM proteins in an integrin-dependent manner to promote infiltration.

Integrin αvβ3, consisting of αv and β3 subunits, binds to RGD-containing ECM proteins to transduce biochemical and mechanical signals between cells and extracellular environments. It is highly expressed in invasive tumor cells and exerts a tumor-promoting effect on various cancers, including HCC.[8] Integrin αvβ3 inhibitors have demonstrated promising antitumor activities in preclinical studies, but failed in clinical trials.[9] Further investigations have revealed that integrin αvβ3 plays an important role in non-tumor cells. Integrin β3-null mural cells interact with tumor cells to increase tumor burden in mouse models.[10] Integrin αvβ3 is also expressed in immune cells and has the potential to regulate immune functions. Macrophage lineage-specific integrin β3 knockout accelerates tumor growth in murine melanoma and breast cancer models.[11] T cells are major players in the antitumor immune response, and the expression and potential role of integrin αvβ3 in T cells of HCC patients remain largely unknown.

In this study, we collected peripheral blood as well as tumor and non-tumor tissues from HCC patients to examine integrin αvβ3 expression on T cells, and investigated the role of integrin αvβ3 in T cell-mediated antitumor immunity in vivo and

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in vitro. Furthermore, we explored whether platelets regulate T cell immune response via integrins.

Materials and methods

Mice and tumor models

Details are described in supplemental materials.

In vivo bioluminescence imaging

To monitor tumor burden, mice were intraperitoneally injected with 150 mg/kg D-luciferin potassium salt (40902ES02, Yeasen). After 5 mins, mice were anesthetized with isoflurane and imaged by the *IVIS® Spectrum* Imaging System. The bioluminescent signal was measured in the region of interest to estimate tumor burden. Detailed are described in supplemental materials.

Patients and samples

Fresh paired tumor and non-tumor tissues were obtained during surgical resection from 30 treatment-naive HCC patients. Table S1 showed the clinical characteristics of patients. Fresh peripheral blood was collected from these HCC patients and 30 healthy donors. Samples were collected at the First Affiliated Hospital of Xi'an Jiaotong University.

Isolation of lymphocytes from peripheral blood, and tumor and non-tumor tissues

Details are described in supplemental materials.

Absolute counting of infiltrating T cells

The isolated lymphocytes were stained with fluorochrome-conjugated antibodies. Absolute Counting Beads (C36950, Invitrogen) were then added to quantify stained cells by flow cytometry using the following formula:

$$Absolute\ counting\ (cells) = \frac{\text{Stained cells count} \times \text{Counting beads volume}}{\text{Counting beads count}} \times \text{Counting beads concentration}$$

Flow cytometry, immunofluorescence (IF), and immunohistochemistry (IHC)

Details are described in supplemental materials.

Quantitative real-time PCR (qRT-PCR) and western blotting (WB)

Details are described in supplemental materials.

Measurement of cytokines and chemokines by Luminex assays

Cell Lysis Buffer 2 (895347, R&D Systems) was used to extract total protein from subcutaneous tumors of BALB/c mice, and 433 μg total protein in 100 μL from each sample was analyzed. Quantification of cytokine concentrations was performed using the LXSAMSM-23 Mouse Luminex Discovery Assay (R&D Systems) in accordance with the manufacturer's instructions. Mean fluorescence intensity (Y-axis) was measured using Luminex 200. The protein concentration (x-axis) was calculated using a five-parameter standard curve. Formula: Y=a+((b-a)/(1+((x/c) ^d)) ^f). Cytokine and chemokine levels were normalized to per mg total protein. Detailed are described in supplemental materials.

Isolation and culture of human primary T cells

Details are described in supplemental materials.

F-actin staining

Details are described in supplemental materials.

Co-culture of T cells with platelets or platelet-derived microparticles (PMPs)

Details are described in supplemental materials.

Cellular uptake of PMPs and endocytosis inhibition assay

Details are described in supplemental materials.

Statistical analysis

Quantitative variables are described as the mean±SEM and were compared using Student's *t*-test unless indicated otherwise. The Pearson test was used for correlation analysis. $P < 0.05$ was considered statistically significant.

Study approval

This study was approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University, China (Approval No. 2019-G-201). Written informed consent was obtained from HCC patients and healthy volunteers prior to inclusion in the study. Animal welfare and experimental procedures were performed in accordance with the guidelines established by the Institutional Animal Care Committee of Xi'an Jiaotong University. All efforts were made to reduce the number of animals used and minimize animal suffering.

Results

Integrin αvβ3 plays a dual role in tumor progression and T cell immune response

To investigate the role of integrin αvβ3 in T cell immune response against HCC, we established HCC models in BALB/c nude mice and BALB/c mice, respectively. The therapeutic effects of cilengitide, an integrin αvβ3 inhibitor, were then compared.

In BALB/c nude mice, cilengitide significantly decreased tumor burden (Figure 1b-d), and tumor weight (Figure 1e,f). Kaplan – Meier survival analysis revealed prolonged survival of

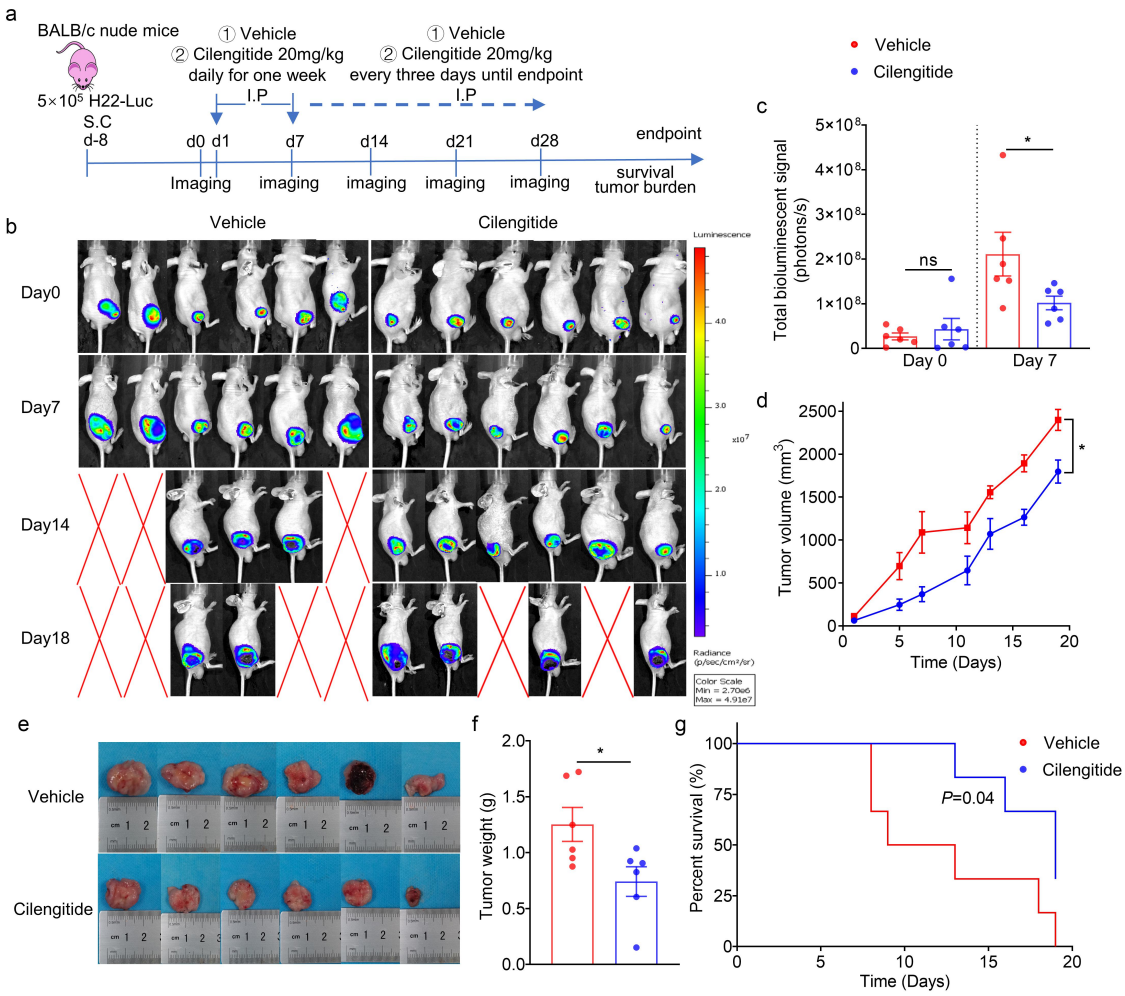

Figure 1. Antagonizing integrin αvβ3 inhibits HCC growth and prolongs survival of T cell-deficient mice. (a) Schema of experimental design in BALB/c nude mice. (b) Tumors were visualized and (c) quantified by BLI. (d) Tumor volumes were measured with a caliper. (e) Isolated tumors were photographed and (f) weighted. (g) Kaplan–Meier survival plot. BLI, bioluminescence imaging; I.P, intraperitoneal; S.C, subcutaneous. $n = 6$; *$P < 0.05$; ns, $P > 0.05$.

the cilengitide group (Figure 1g). Furthermore, cilengitide suppressed tumor cell proliferation with fewer Ki-67-positive cells, but did not affect their apoptosis with comparable numbers of TUNEL-positive cells. (Figure S1A). Cilengitide reduced tumor burden and improved overall survival, which may be ascribed to the reduced proliferative activity of tumor cells. In BALB/c mice, tumor burden in the cilengitide group was comparable to that in the control group (Figure 2b-d). BALB/c mice did not benefit from cilengitide treatment, even an increasing trend in tumor weight was noted (Figure 2e,f). Although cilengitide also significantly inhibited tumor cell proliferation (Figure S1B).

In the two HCC models, the discrepant responsiveness to cilengitide treatment suggests that integrin αvβ3 plays a dual role in tumor progression and T cell immune response.

Antagonizing integrin αvβ3 attenuates T-cell infiltration and Th1-type immune response in the TME

In BALB/c mice, we further investigated the immune landscape of T cells in the TME. Cilengitide treatment reduced the number of tumor-infiltrating $CD45^+$ leukocytes and $CD3^+$ T cells, particularly cytotoxic $CD8^+$ T cells and Th1-type $CD4^+$ T cells (Figure 3c), while the quantity of immune cells in peripheral blood was unaffected (Figure 3b). IF staining confirmed a significant decrease in tumor-infiltrating $CD3^+$ and $CD8^+$ T cells in the cilengitide group (Figure 3d). Cilengitide treatment did not affect the secretion of T-cell chemokines, such as CCL2, CCL3, CCL4, CCL5, and CCL21, implying that the decreased T-cell infiltration may be independent of chemokines (Figure 3g). Furthermore, cilengitide treatment downregulated mRNA expression of *Gzmb*, *Ifng*, *Tbx21*, and *Foxp3* in tumors (Figure 3e), whereas granzyme B infiltration and FOXP3-positive cells were unaltered in IHC staining (Figure S1C). After cilengitide treatment, IFN-γ level in tumor homogenates was decreased. Th2-related cytokines levels in tumor homogenates, such as IL-4, IL-5, IL-6, IL-10, and IL-13, remained unchanged (Figure 3f). We unexpectedly found that GM-CSF and CXCL1 levels were significantly decreased after cilengitide treatment, suggesting that integrin αvβ3 may regulate the function of myeloid cells. Additionally, cilengitide treatment did not affect T-cell exhaustion (Figure S1E, S1F).

These results indicate that antagonizing integrin αvβ3 decreases T-cell infiltration and impairs Th1-type immune response, contributing to the formation of an immunosuppressive TME.

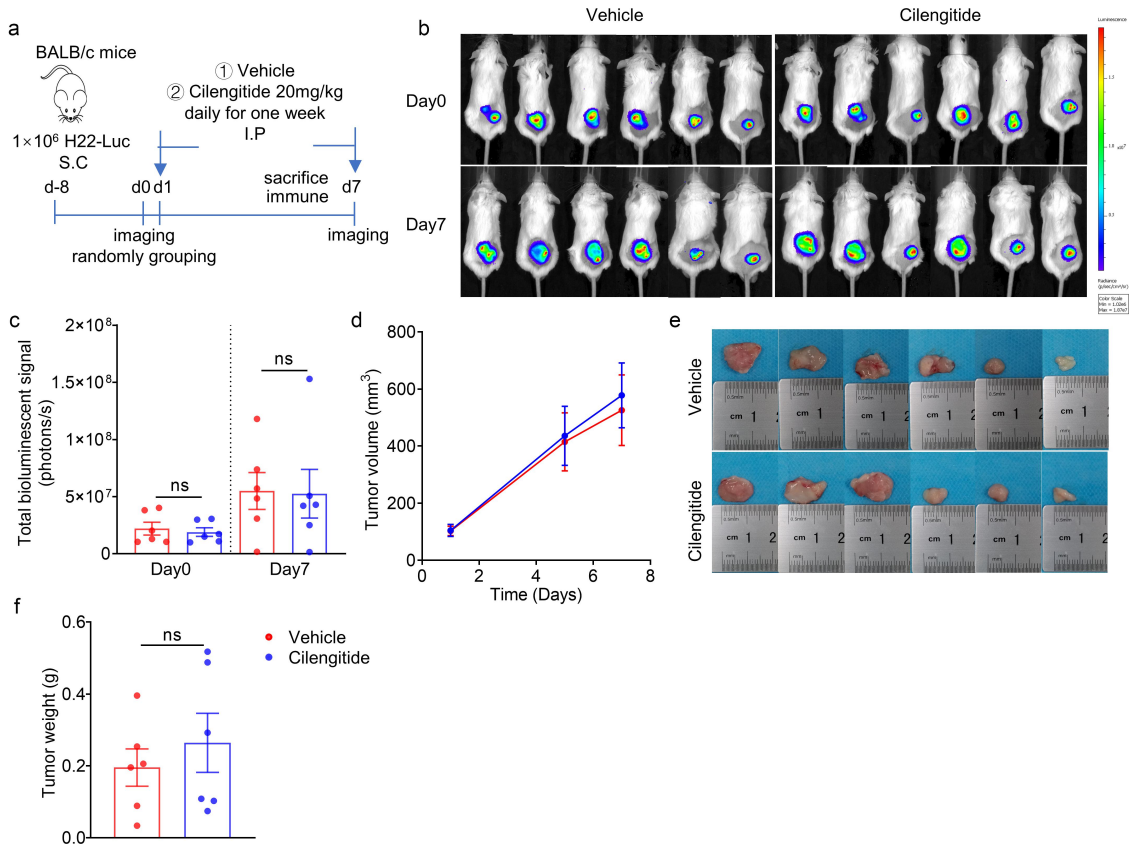

Figure 2. Antagonizing integrin αvβ3 does not inhibit HCC growth in immunocompetent mice. (a) Schema of experimental design in BALB/c mice. (b) Tumors were visualized and (c) quantified by BLI. (d) Tumor volumes were measured with a caliper. (e) Isolated tumors were photographed and (f) weighted. $n = 6$; ns, $P > 0.05$.

HCC-infiltrating T cells exhibit deficient expression and activation of integrin β3 (ITGB3), and expression of ITGB3, but not integrin av (ITGAV), is positively correlated with the number of infiltrating T cells

To clarify integrin αvβ3 expression on T cells in HCC patients and explore its potential clinical implications, we collected peripheral blood, tumor tissue, and non-tumor tissue from HCC patients and isolated the lymphocytes. ITGB3 expression on infiltrating T cells (from tumor or non-tumor tissues) was significantly lower than that on circulating T cells (from peripheral blood), while ITGAV expression was comparable between infiltrating and circulating T cells (Figure 4a,b, Figure S2A). Integrin αvβ3 promotes clustering and activation of naïve T cells, and its expression is further elevated during activation.[12] Upon TCR activation, ITGB3 expression on circulating T cells was upregulated, but its expression on infiltrating T cells was further downregulated (Figure 4c,d), and ITGAV expression remained unchanged both on circulating and infiltrating T cells (Figure S2B, S2C). Moreover, strong positive correlations were found between the numbers of tumor-infiltrating $CD3^+$, $CD4^+$, and $CD8^+$ T cells and ITGB3 expression on these infiltrating T cells (Figure 4e). Patients with $ITGB3^{high}$ expression had more tumor-infiltrating T cells than $ITGB3^{Low}$ patients (Figure 4f). Consistent result was obtained when tumor-infiltrating $CD3^+$ and $CD8^+$ T cells were quantified by IF staining (Figure 4g). However, no correlation was observed between the number of tumor-infiltrating T cells and ITGAV expression (Figure S2D), and patients with $ITGAV^{high}$ expression had comparable tumor-infiltrating T cells with $ITGAV^{Low}$ patients (Figure S2E).

Hence, these data from human specimens suggest that HCC-infiltrating T cells exhibit deficient expression and activation of ITGB3, which is associated with poor T-cell infiltration into the tumor.

ITGB3 promotes T-cell infiltration and Th1-type immune response in vitro

To investigate the role of ITGB3 in T cell immune response, pharmacological blockade or gene overexpression was applied to ITGB3 in vitro. After blockade of ITGB3 signaling by cilengitide, transwell assay revealed that fibronectin-mediated migration of $CD3^+$, $CD4^+$, and $CD8^+$ T cells was decreased obviously (Figure 5a,b), and adhesion assay showed that the number of T cells adhering to coated fibronectin was lower (Figure 5c). Pretreating T cells with cilengitide reduced IFN-γ and IL-2 secretion, but not granzyme B or IL-4 secretion (Figure 5d,e). Also, cilengitide inhibited Th1 polarization of $CD4^+$ T cells, but not Treg or Th17 polarization (Figure 5f, Figure S3A). Moreover, cilengitide suppressed T-cell proliferation, but not T-cell apoptosis or exhaustion (Figure S3B – S3D). Next, we established stable ITGB3-overexpressing Jurkat T cells (Figure 5g,h). Overexpression of ITGB3 increased fibronectin-mediated migration of Jurkat T cells (Figure 5i). Notably, F-actin staining

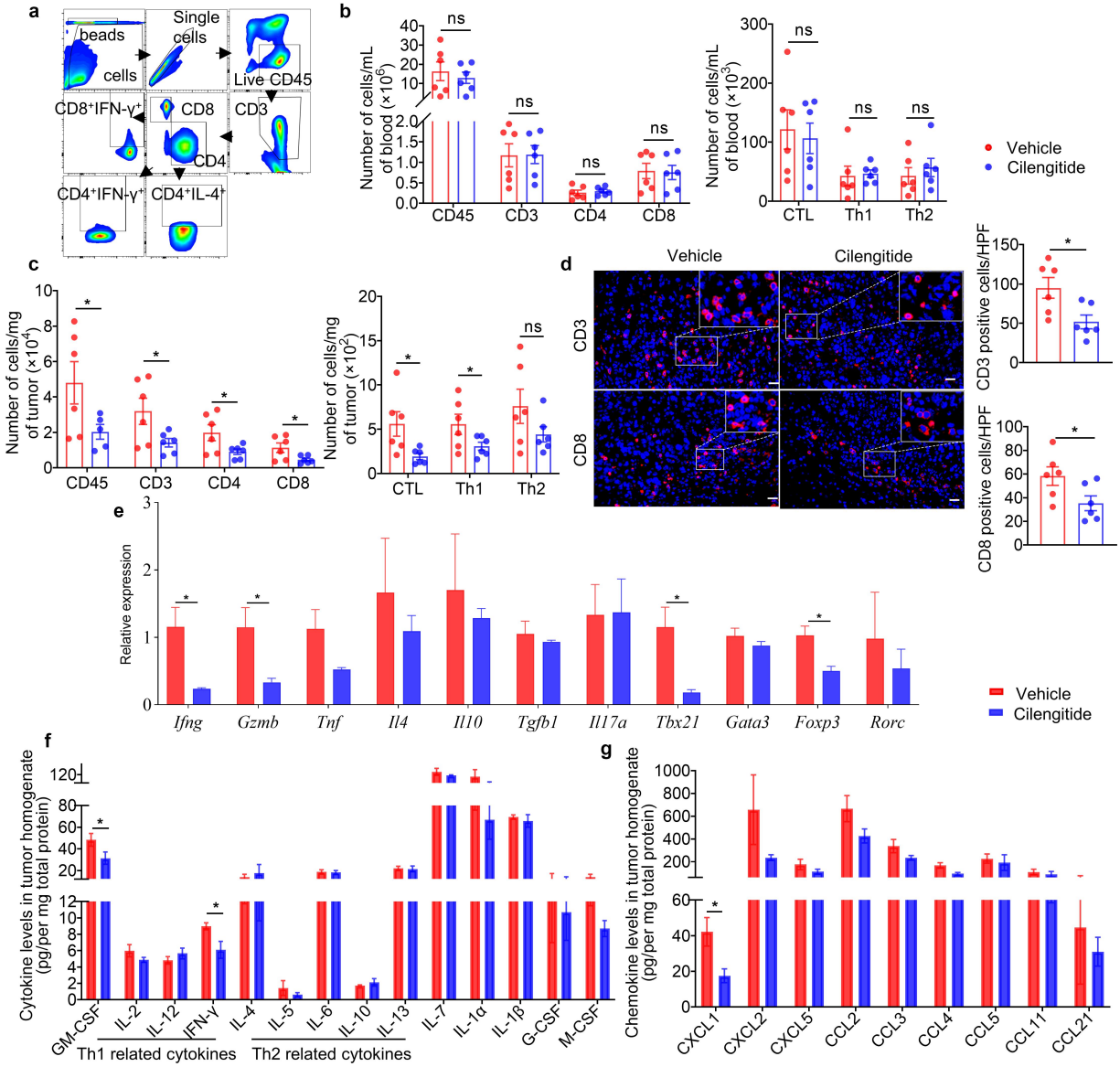

Figure 3. Antagonizing integrin αvβ3 attenuates T-cell infiltration and Th1-type immune response in the TME. The experiments were performed in tumor-bearing BALB/c mice. (a) Gating strategy for flow cytometry. (b) Quantification of immune cells in peripheral blood and (c) tumors by flow cytometry. $n = 6$. (d) Representative photomicrographs and quantification of IF staining with anti-CD3 (red) and anti-CD8 (red) antibodies and DAPI (blue) in tumors. Scale bars, 20 μm; $n = 6$. (e) Relative mRNA expression of Th-related cytokines and transcription factors in tumors. Vehicle, $n = 4$; cilengitide, $n = 3$. (f) Quantification of cytokines and (g) chemokines per mg of tumor homogenate proteins by Luminex assay. Vehicle, $n = 4$; cilengitide, $n = 3$. CTL, cytotoxic T lymphocyte; HPF, high powered field. CTL, $CD8^+IFN\text{-}\gamma^+$; Th1, $CD4^+IFN\text{-}\gamma^+$; Th2, $CD4^+IL\text{-}4^+$. *$P < 0.05$; ns, $P > 0.05$.

showed a cap-like structure in ITGB3-Jurkat T cells and a homogenous distribution in vector-Jurkat T cells (Figure 5j). Overexpression of ITGB3 in Jurkat T cells increased expression of *IFN-γ* and Th1-specific transcription factor TBX21, whereas expression of *IL-4* and Th2-specific transcription factor GATA3 remained unchanged (Figure 5k,l).

Using various strategies to target ITGB3 in vitro, we demonstrate that ITGB3, as a positive immunomodulatory molecule, facilitates T-cell infiltration and Th1-type immune response.

PMPs adoptively transfer ITGB3 to T cells and positively regulate T cell immune response

Platelets are involved in regulation of innate and adaptive immune responses. ITGB3 is mainly expressed in the plasma membrane of platelets. Here, we further explored whether platelets regulate T cell immune response through ITGB3. HCC patients with $ITGB3^{high}$ expression had higher platelet count than $ITGB3^{Low}$ patients, and a positive correlation was found between platelet count and ITGB3 expression on circulating T cells (Figure 6a,b). After co-culture with platelets, ITGB3 expression on T cells was significantly upregulated at the protein level, but not at the transcriptional level (Figure 6c), and its expression was upregulated in a platelet dose-dependent manner (Figure 6d). Unexpectedly, the upregulated ITGB3 expression on T cells was largely attenuated by transwell co-culture with platelets using a 0.4 μm pore size (Figure 6e). Thus, we further explored whether PMPs with diameters ranging from 0.1 to 1.0 μm were involved in this process. The isolated PMPs were identified as shown Figure 6f.

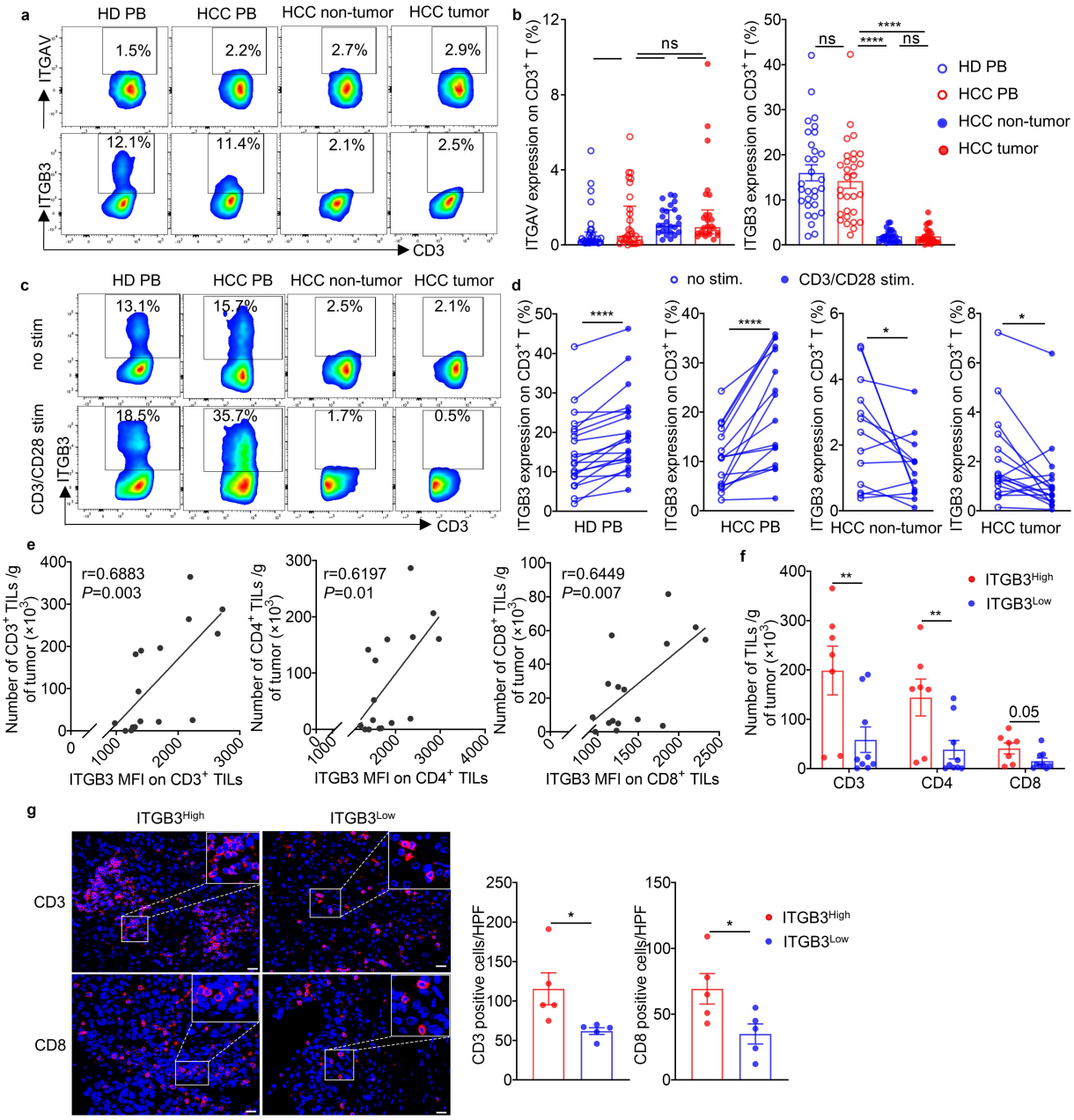

Figure 4. HCC-infiltrating T cells exhibit deficient expression and activation of ITGB3, and expression of ITGB3, but not ITGAV, is positively correlated with the number of infiltrating T cells. (a, b) Expression of ITGAV and ITGB3 on T cells in HD PB ($n = 30$), HCC PB ($n = 30$), HCC non-tumor tissue ($n = 26$), and HCC tumor tissue ($n = 27$). (a) Representative flow cytometry plots. (b) Statistical results. (c, d) ITGB3 expression on resting vs. activated T cells. HD PB ($n = 20$), HCC PB ($n = 16$), HCC non-tumor tissue ($n = 13$), and HCC tumor tissue ($n = 17$). (c) Representative flow cytometry plots. (d) Statistical results. Paired *t*-test. (e) Correlation analysis between ITGB3 expression on TILs and the number of TILs in human HCC tumors ($n = 16$). (f, g) HCC patients were divided into $ITGB3^{High}$ and $ITGB3^{Low}$ groups by ITGB3 expression on $CD3^+$ TILs. (f) The number of infiltrating T cells in tumors was compared using flow cytometry ($n = 16$) and (g) IF staining. Representative photomicrographs and quantification of IF staining with anti-CD3 (red) and anti-CD8α (red) antibodies and DAPI (blue) in tumors. Scale bars, 20 μm; ($n = 10$). HD, healthy donor; HCC, hepatocellular carcinoma; PB, peripheral blood; MFI, mean fluoresce intensity; ITGAV, integrin αv; ITGB3, integrin β3. TILs, tumor-infiltrating lymphocytes. $^*P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.001$; ns, $P > 0.05$.

Similarly, ITGB3 expression on T cells was upregulated only at the protein level after incubation with PMPs (Figure 6g,h). Confocal imaging demonstrated uptake of PMPs by T cells (Figure 6i). Chlorpromazine and amiloride significantly inhibited uptake of PMPs by T cells, whereas methyl-β-cyclodextrin barely affected cellular entry of PMPs (Figure 6j). After co-culture with PMPs, T cells had an increased migratory capacity toward fibronectin (Figure 6k), and $CD4^+$ T cells preferred to polarize toward Th1 cells instead of Th2 and Treg cells (Figure 6l).

Overall, our results indicate that PMPs adoptively transfer ITGB3 to T cells and positively regulate T cell immune response, and this process is mediated by clathrin-dependent endocytosis and macropinocytosis (Figure 7).

Discussion

We found that cilengitide had a dual effect on the HCC microenvironment by exerting both antitumor effect and immunosuppressive effect on T cells. In clinical specimens, HCC-

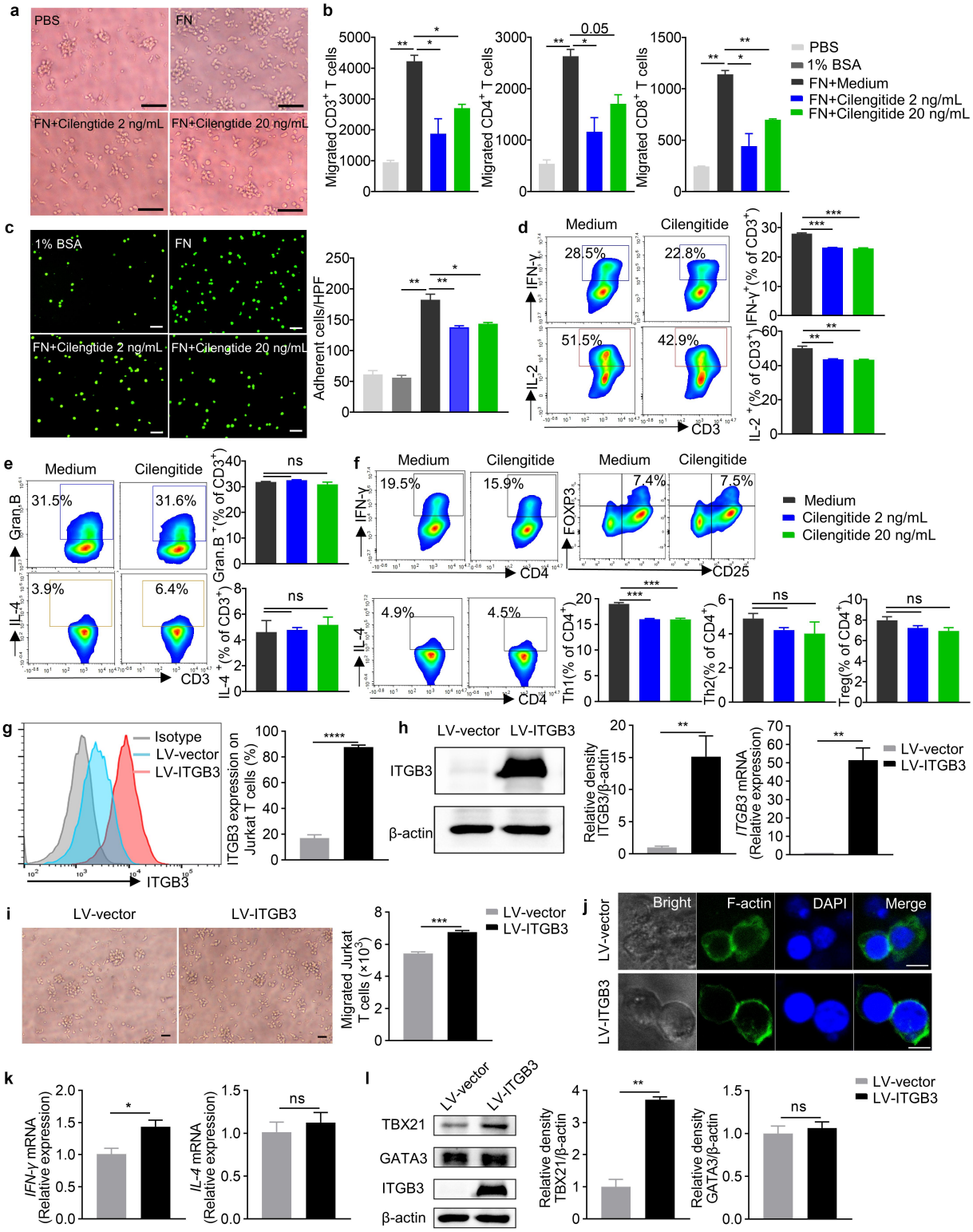

Figure 5. ITGB3 promotes T-cell infiltration and Th1-type immune response in vitro. (a, b) Transwell assay. (a) Migrated T cells in the lower chamber were photographed and (b) quantified by flow cytometry. Scale bars, 50 μm. (c) Adhesion assay. Representative photomicrographs of CFSE-labelled T cells adhering to 1% BSA or FN. Scale bars, 50 μm (left). statistical results (right). (d, e) Representative flow cytometry plots of IFN-γ-, IL-2-, granzyme B-, IL-4-positive $CD3^+$ T cells, and statistical results. (f) Representative flow cytometry plots of Th1, Th2, and Treg cells, and statistical results. (g, h) ITGB3 overexpression in Jurkat T cells was confirmed by (g) flow cytometry (h) western blotting, and qRT-PCR. (i) Transwell assay. Migrated Jurkat T cells were photographed and quantified by flow cytometry. Scale bars, 20 μm. (j) Representative confocal images of F-actin (green), nuclei (blue). Scale bars, 5 μm. (k) Relative mRNA expression of *IFN-γ* and *IL-4* in Jurkat T cells. (l) WB analysis of TBX21 and GATA3 protein levels in Jurkat T cells, and statistical results. FN, fibronectin; Gran.B, granzyme B. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; ns, $P > 0.05$.

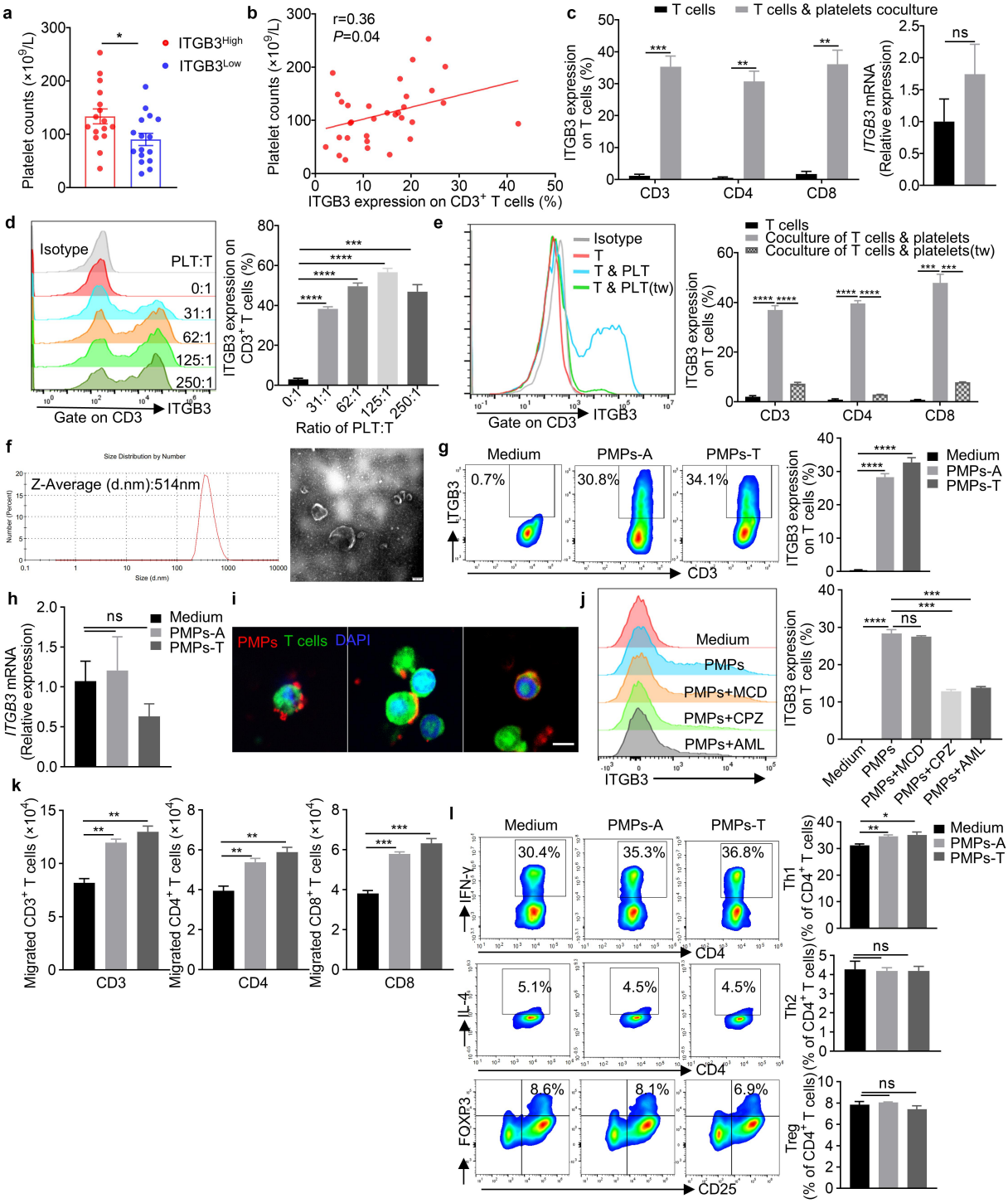

Figure 6. PMPs adoptively transfer ITGB3 to T cells and positively regulate T cell immune response. (a) HCC patients were divided into ITGB3^{High} and ITGB3^{Low} groups by ITGB3 expression on CD3$^+$ T cells in peripheral blood, and platelet counts were compared ($n = 32$). (b) Correlation analysis between platelet counts and ITGB3 expression on CD3$^+$ T cells in peripheral blood ($n = 32$). (c) ITGB3 expression on T cells was evaluated after co-culture with platelets at a ratio of 1:250 by flow cytometry (left) and qRT-PCR (right) ($n = 5$). (d) ITGB3 expression on T cells after co-culture with platelets at various ratios. Representative histogram overlay (left) and statistical results (right). (e) ITGB3 expression on T cells after direct co-culture and transwell co-culture with platelets at a ratio of 1:250. Representative histogram overlay (left) and statistical results (right). (f) Identification of PMPs. Particle size analysis (left) and representative transmission electron micrograph (right). Scale bars, 200 nm. (g) ITGB3 expression on T cells was evaluated after co-culture with PMPs by flow cytometry and (h) qRT-PCR. (i) Representative confocal images of PMPs (red) uptake by T cells (green), nuclei (blue). Scale bars, 5 μm. (j) Quantification of PMPs uptake by T cells after inhibiting various endocytic pathways. Representative histogram overlay (left) and statistical results (right). (k) Transwell assay after co-culture with PMPs. Migrated T cells were quantified by flow cytometry. (l) CD4$^+$ T-cell subsets were evaluated after co-culture with PMPs. Representative flow cytometry plots of Th1, Th2, and Treg cells (left) and statistical results (right). tw, transwell; MCD, methyl-β-cyclodextrin; CPZ, chlorpromazine; AML, amiloride; PMPs, platelet-derived microparticles; PMPs-A (activated by ADP); PMPs-T (activated by trap-6). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; ns, $P > 0.05$.

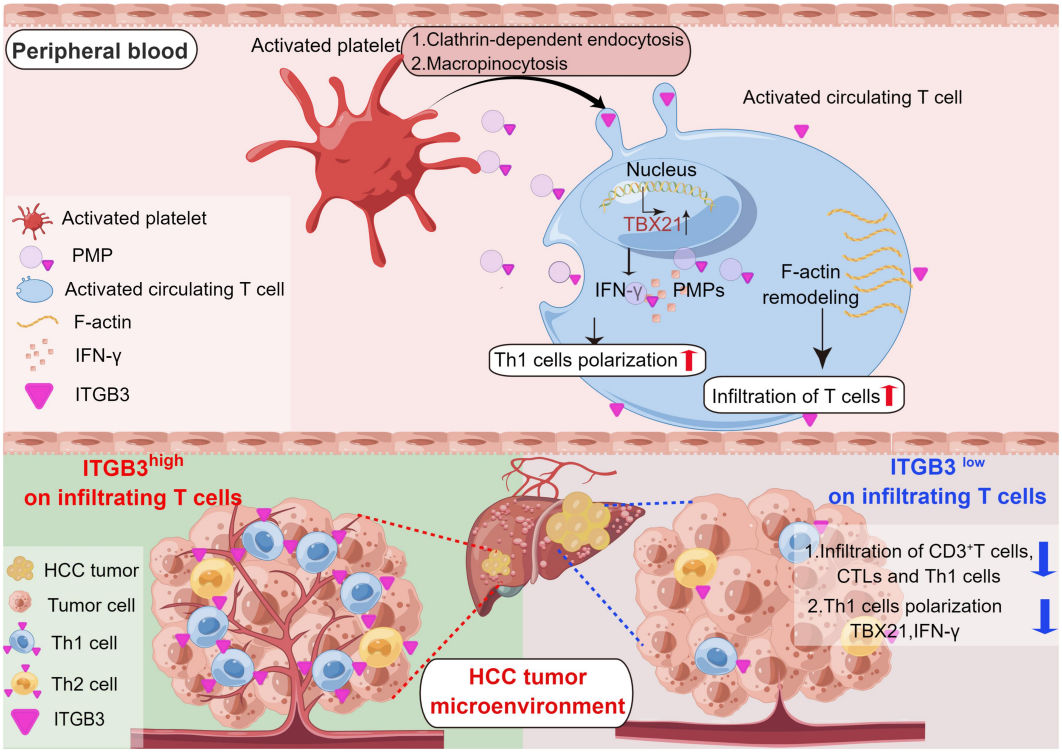

Figure 7. Platelet-derived microparticles adoptively transfer integrin β3 to promote antitumor effect of tumor-infiltrating T cells in HCC. In peripheral blood, activated platelets produce abundant ITGB3-containing microparticles. These microparticles are taken up by activated T cells via clathrin-dependent endocytosis and macropinocytosis. Microparticles-delivered ITGB3 promotes T-cell infiltration and Th1-type immune response. In the TME, HCC-infiltrating T cells exhibit deficient expression and activation of ITGB3, which is associated with poor T-cell infiltration into the tumor. Pharmacological blockade of ITGB3 signaling impaired T-cell infiltration and Th1-type immune response.

infiltrating T cells exhibited deficient expression and activation of ITGB3, which was associated with poor T-cell infiltration into the tumor. Additionally, ITGB3 functioned as a positive immunomodulatory molecule to facilitate T-cell infiltration and Th1-type immune response. Notably, PMPs adoptively transferred ITGB3 to T cells and positively regulated T cell immune response.

In line with a previous study,[13] we revealed that cilengitide exerted a therapeutic effect in tumor-bearing T cell-deficient mice. However, tumor-bearing immunocompetent mice were unable to benefit from cilengitide treatment, and the immune landscape of T cells in the TME was characterized by decreased T-cell infiltration and an impaired Th1-type immune response. This suggests that cilengitide treatment contributes to the formation of an immunosuppressive TME. The discrepant responsiveness in the two HCC models suggests that cilengitide treatment exerts a dual effect on the TME by exerting both antitumor effect and immunosuppressive effect on T cells. These results provide an explanation for the failures of cilengitide in clinical trials from the perspective of T cells.

In clinical HCC specimens, we observed that ITGB3 expression on infiltrating T cells was significantly lower than that on circulating T cells, while ITGAV expression was comparable on infiltrating T cells and circulating T cells. This suggests that changes in ITGAV expression do not always parallel concomitant changes in ITGB3 expression, implying that it may be less comprehensive to study integrin αvβ3 as a whole. Certain ECM proteins in TME may be responsible for the decreased ITGB3 expression on T cells.[14] Previous studies have revealed that TGFβ1[15] and MMP8[16] negatively regulate ITGB3 expression. Thus, we treated T cells with corresponding recombinant proteins, but ITGB3 expression on T cells was unaltered (data not shown). Further investigation is needed to elucidate the specific mechanism. In line with a previous study,[12] ITGB3 expression on circulating T cells was upregulated upon TCR activation. This may be because platelets adoptively transferred ITGB3 to circulating T cells via PMPs. Conversely, ITGB3 expression on infiltrating T cells decreased rapidly upon activation. This might be attributed to re-localization of ITGB3.[14,17]

Previous studies mainly focusing on inflammatory diseases have reported an inconsistent role of integrin αvβ3 in T cell immune response. Several reports have emphasized that integrin αvβ3 facilitates T cell migration into inflamed tissue.[7,15,18] However, Larochelle et.al have reported that EGFL7 binds to integrin αvβ3 to restrict T-cell infiltration into an inflamed brain.[12] These conflicting findings may be attributed to heterogeneous ECM proteins in various diseases. In the context of HCC, we demonstrated that ITGB3 functioned as a positive immunomodulatory molecule to facilitate T-cell infiltration and Th1-type immune response. This suggests that ITGB3 deficiency on HCC-infiltrating T cells may be involved in shaping the immunosuppressive TME. Overexpression of ITGB3 in Jurkat T cells promoted polar accumulation of F-actin. This dynamic remodeling of F-actin provides the impetus for cell invasion and migration.[19,20]

Platelets communicate with T cells via multiple soluble mediators and surface adhesion receptors, performing either immunosuppressive[21,22] or immunostimulatory[23,24] function. Our study revealed that platelets positively regulated T cell immune response via PMPs. PMPs package and transfer bioactive molecules to nucleated recipients and regulate the function of target cells.[25] We initially found that PMPs adoptively transferred ITGB3 to T cells, and this process was mediated by clathrin-dependent endocytosis and macropinocytosis. Additionally, the microparticle-delivered ITGB3 promoted T-cell infiltration and Th1-type immune response. These results provide a theoretical basis for the immunomodulatory effect of PMPs.

Collectively, our study demonstrates that ITGB3 functions as a positive immunomodulatory molecule to facilitate T-cell infiltration and Th1-type immune response in the HCC microenvironment. HCC-infiltrating T cells exhibit deficient expression and activation of ITGB3, which may be involved in shaping an immunosuppressive TME. Notably, PMPs adoptively transfer ITGB3 to T cells and positively regulate T cell immune response, which may provide a new insight into immune therapy of HCC.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

All data generated in this study are available from the corresponding author upon reasonable request.

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