

IL-17B Inhibits Hepatocellular Carcinoma Cell Proliferation

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Abstract

IL-17RB is a cytokine receptor that binds interleukin (IL)-17B and IL-17E. While analyzing IL-17RB expression in various cancer cell lines, we found that this receptor is highly expressed at both the mRNA and protein levels in hepatocellular carcinoma (HCC) cells. This finding prompted us to investigate the effects of its ligand, IL-17B, on the proliferation of these cells. Our results demonstrate that IL-17B inhibits the proliferation of HCC cells through an AKT-dependent, but NF- κ B-independent, mechanism. Additionally, IL-17B affected colony formation in these cells. Interestingly, these effects were not observed in melanoma cells, which express low levels of IL-17RB. Our results may represent a promising new approach for treating HCC – a disease for which immunological therapies have recently gained significant attention – especially considering that HCC patients experience progressive liver dysfunction and that the IL-17B–IL-17RB signaling pathway plays a role in liver regeneration.

Keywords

IL-17B · IL-17RB · Hepatocellular carcinoma · Proliferation

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1. Introduction

Interleukin (IL)-17 is a key cytokine with a well-established role in regulating immune system function. To date, six members of the IL-17 superfamily have been identified in humans: IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F (Moseley et al. 2003; Zenobia and Hajishengallis 2015). While IL-17A has been extensively studied, research on IL-17B and IL-17E remains limited. However, emerging evidence suggests that both cytokines may play a role in cancer progression and metastasis. IL-17B has been shown to stimulate the production of IL-6 and granulocyte colony-stimulating factor in fibroblasts, as well as that of tumor necrosis factor (TNF)- α and IL-1 β in monocytic cells. Additionally, it influences the migration of germinal center B cells toward CXCL12 and CXCL13 and promotes neutrophil recruitment to the peritoneal cavity (Bie et al. 2017a). Similarly, IL-17E has been found to activate the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) signaling pathway and subsequently stimulate CXCL8 (IL-8) release from kidney-derived cells (Lee et al. 2001). Moreover, this cytokine suppresses matrix synthesis, induces nitric oxide release, and promotes IL-6 production (Cai et al. 2001). Both IL-17B and IL-17E act as ligands for IL-17 receptor B (IL-17RB) (Shi et al. 2000; Lee et al. 2001). Signaling mediated by this receptor and its ligands has been linked to multiple types of cancer, including

breast cancer (Huang et al. 2014; Jiang et al. 2017), gastric cancer (Bie et al. 2016, 2017b), pancreatic cancer (Wu et al. 2015), thyroid cancer (Ren et al. 2017), and lung cancer (Yang et al. 2018). High IL-17RB expression in cancer cells may also have potential therapeutic implications. Furuta et al. (2011) demonstrated that IL-17E induces caspase-mediated apoptosis in breast cancer cells with high IL-17RB expression. Additionally, Benatar et al. (2010) reported the antitumor activity of IL-17E in models of human melanoma and pancreatic, colon, lung, and breast cancers, whereas Lemancewicz et al. (2012) reported similar effects in a multiple myeloma model.

In this study, we examined IL-17RB expression across various cancers originating from different tissues and found particularly high expression in hepatocellular carcinoma (HCC) cell lines. This finding led us to investigate the effect of IL-17B on these cells, revealing that the cytokine inhibits their proliferation in an NF- κ B-independent manner. Furthermore, RNA sequencing (RNA-seq) analysis of cytokine-treated cells revealed that IL-17B influences the expression of genes related to cation transport, potassium channels, and nonsense-mediated mRNA decay (NMD). Our findings may contribute to the development of novel immunotherapeutic strategies targeting the tumor micro-environment of HCC, potentially enhancing the effectiveness of currently employed treatment regimens for HCC patients.

2. Materials and Methods

2.1. Reagents

Recombinant IL-17B was purchased from PeproTech (Rocky Hill, NJ, USA). TNF- α was purchased from Merck (Darmstadt,

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Germany). The IL-17RB-blocking peptide was purchased from St John's Laboratory (London, UK).

2.2. Cell culture

All the cell lines used in this study were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained under standard conditions at 37°C in a 5% CO₂ atmosphere. HepG2 (HCC), Hep3B (HCC), A375 (malignant melanoma), HeLa (cervical adenocarcinoma), A549 (lung carcinoma), HEK293 (immortalized embryonic kidney), and MCF7 (breast adenocarcinoma) cells were cultured in DMEM (PAN Biotech GmbH, Aidenbach, Germany). Jurkat (T lymphocytes) and LNCaP (prostate carcinoma) cells were grown in RPMI 1640 (PAN Biotech GmbH), while K562 (chronic myeloid leukemia) cells were cultured in IMDM (PAN Biotech GmbH) supplemented with 10% charcoal-stripped fetal bovine serum (FBS; PAN Biotech GmbH).

2.3. Real-time quantitative PCR (RT-qPCR)

Total RNA was isolated using TRIzol Reagent (Molecular Research Center, Cincinnati, OH, USA) and resuspended in nuclease-free water. For cDNA synthesis, 5 µg of RNA was used as input, and a Maxima First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA) was used following the manufacturer's instructions. RT-qPCR was carried out using the LightCycler 480 platform (Roche, Basel, Switzerland) with SYBR Green I Master Mix (Roche) as the detection reagent. The PCR protocol consisted of initial denaturation (95°C, 5 min), followed by 40 cycles of denaturation (95°C, 10 s), annealing (60°C, 10 s), and elongation (72°C, 20 s). The primer sequences for *IL17RB* detection were as follows: forward, 5'-ATCCCCGGAGACTTGAGG-3'; and reverse, 5'-CAGGGAAGCCGATGTAGG-3', while for *IL6* detection were as follows: forward, 5'-CCTGAACCTTCCAAAGATGG-3'; and reverse, 5'-GGTCAGGGGTGGTTATTGC-3'. The mRNA levels were normalized to the geometric means of three housekeeping genes: ribosomal protein L13A (*RPL13A*), hydroxymethylbilane synthase (*HMBS*), and hypoxanthine phosphoribosyltransferase 1 (*HPRT1*), following the methods described by Vandesompele et al. (2002).

2.4. Western blotting

For the analysis of IL-17RB expression, cells were seeded in 6-well plates at a density of 0.5×10^6 cells per well. After 48 h, the cells were harvested and lysed as previously described (Karwaciak et al. 2019). For the analysis of phosphorylated IκBα and AKT (RAC-α serine/threonine-protein kinase), HepG2 cells were seeded at the same density (0.5×10^6 cells per well). After 24 h, the cells were treated with increasing

concentrations of IL-17B for an additional 24 h before being harvested and lysed.

The following antibodies were used: anti-IL17RB (No. 144-10147; RayBiotech, Peachtree Corners, GA, USA); anti-*plkBα* (No. 9246) and anti-IκBα (No. 4812); anti-β-actin (No. 4970) from Cell Signaling Technology (Danvers, MA, USA); anti-pAKT (No. GTX128414); and anti-AKT (No. GTX110613) from GeneTex (Alton Pkwy, Irvine, CA, USA). Detection was achieved using an HRP-linked secondary antibody (Ab6721, Abcam, Cambridge, UK), and signal development was performed with the SuperSignal West Pico chemiluminescent substrate (Thermo Fisher Scientific). The protein bands were visualized using a G:Box imaging system (Syngene, Cambridge, UK).

2.5. BrdU proliferation assay

HepG2, Hep3B, and A375 cells were seeded into 96-well transparent plates at a density of 2.5×10^3 cells per well. After 24 h, the cells were treated with increasing concentrations of IL-17B. They were then cultured for an additional 96 h before a BrdU proliferation assay was performed using a BrdU Cell Proliferation ELISA Kit (Ab126556, Abcam), following the manufacturer's instructions, as previously described (Karaś et al. 2023). Heat inactivation of IL-17B was carried out in a thermocycler at 99°C for 1 h. The absorbance was measured at 450/550 nm using an Infinite® 200 PRO microplate reader (Tecan, Männedorf, Switzerland).

2.6. Colony-forming assay

To assess colony formation, HepG2 and A375 cells were seeded into 6-well plates at densities of 10×10^4 and 1×10^3 cells per well, respectively. After 24-h incubation, the cells were treated with IL-17B (750 ng/mL) and maintained for 21 days (HepG2) or 10 days (A375). The culture medium and IL-17B treatment mixture were changed every 4 days. After the indicated time, the cells were fixed with 100% methanol for 20 min. Colonies were rinsed, stained with 0.5% crystal violet (in 25% methanol) for 5 min, washed, and air-dried overnight (Crowley et al. 2016). Images were captured using a G-BOX system (Synoptics, Cambridge, UK) and analyzed via the ImageJ (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, University of Wisconsin, Madison, WI, USA) (v1.53d) ColonyArea plugin (Guzmán et al. 2014).

2.7. Transient transfection and luciferase assay

To investigate whether IL-17B induces an NF-κB response in HepG2 cells, we generated an NF-κB-responsive reporter plasmid. This construct contained six copies of a consensus NF-κB binding site (5'-GGGAATTTCC-3') and a minimal EB1 core promoter, which was subsequently

cloned and inserted into the pGL4.10 plasmid (Promega, Fitchburg, WI, USA).

HepG2 cells were seeded into 96-well white plates at a density of 10×10^3 cells per well. After 24 h, the cells were cotransfected with the NF- κ B reporter vector and pCMV-SEAP (a generous gift from Dr. Schlatter, Zurich, Switzerland) using the TurboFect Transfection Reagent (Thermo Scientific). Following transfection, the cells were treated with increasing concentrations of IL-17B for an additional 24 h.

After treatment, the cells were harvested and lysed, and the luciferase activity in the lysates was measured using an Infinite® 200 PRO system (Tecan). D-luciferin (Cayman Chemical, Ann Arbor, MI, USA) was used as the substrate. To assess the transfection efficiency, alkaline phosphatase activity was measured indirectly with a spectrophotometer at 405 nm.

2.8. RNA-seq and data analysis

Global gene expression changes in HepG2 cells treated with 750 ng/mL of IL-17B ($n = 4$) for 24 h were analyzed using high-resolution RNA-seq at Novogene (Cambridge, UK). The procedures for mRNA isolation and library preparation were previously described (Karaś et al. 2023).

Differential expression analysis was performed using the DESeq2R packages (v1.20.0) (GNU Lesser General Public License); Love et al. 2014). p values were adjusted for multiple testing via the Benjamini–Hochberg method to control the false discovery rate. Genes were considered differentially expressed if they met the criteria of $|\log_2(\text{fold change})| \geq 1$ and an adjusted p value of ≤ 0.05 , as previously described (Karwaciak et al. 2019). The RNA-seq data have been deposited in the NCBI Short-Read Archive under BioProject accession number PRJNA1247794: <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1247794>. Gene Ontology (GO) analysis was conducted using (South Dakota State University, Brookings, SD, USA) (Ge et al. 2020), whereas Reactome pathway analysis was performed using the Reactome database (<http://www.reactome.org>).

2.9. Human monocyte isolation and viability assessment

Peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats using Ficoll density gradient centrifugation. The blood was obtained as anonymized waste material from healthy donors through the Regional Center for Blood Donation and Blood Treatment in Łódź, Poland. Monocytes were subsequently purified using the Classical Monocyte Isolation Kit, human (Miltenyi Biotec, Cat. No. 130-117-337, Bergisch Gladbach, Germany). Isolated cells were cultured in RPMI 1640 medium supplemented with 10% Fetal Bovine Serum (FBS) and 10% human AB serum (PAN Biotech, Aidenbach, Germany). Monocyte viability following IL-17B treatment was evaluated using the CellTiter-Glo®

Luminescent Cell Viability Assay (Promega), which measures cellular ATP levels as an indicator of metabolically active cells. Monocytes were seeded in white 96-well plates at a density of 300,000 cells per well and treated with increasing concentrations of IL-17B for 48 h. After incubation, cells were lysed according to the assay protocol, and luminescence was recorded using the Infinite® 200 PRO plate reader (Tecan).

2.10. Statistics

Statistical analysis was performed using Analysis of Variance (ANOVA) or repeated measures ANOVA, followed by the Student–Newman–Keuls *post hoc* test in SigmaStat v4.0 (Systat Software, Inc., San Jose, CA, USA). A p value of < 0.05 was considered statistically significant.

3. Results and Discussion

We analyzed *IL17RB* expression across various cell lines. Quantitative PCR revealed the highest *IL17RB* mRNA expression in the HepG2 and Hep3B HCC cell lines, with very low expression in A375 and Jurkat cells (Figure 1a). Western blot analysis confirmed these results, with the highest IL-17RB expression in HepG2 cells, lower levels in Hep3B cells, and the lowest IL-17RB expression in A375 and Jurkat cells (Figures 1b and 1c). Since several ILs from the IL-17 family have been shown to influence cancer cell proliferation – both positively and negatively – we investigated whether the IL-17RB ligand IL-17B affects the proliferation of HCC cell lines, which exhibit increased IL-17RB expression compared with other cell types. Bromodeoxyuridine (BrdU) proliferation assays revealed that IL-17B inhibited the proliferation of HepG2 and Hep3B cells by approximately 44% and 33%, respectively, at the highest concentration. However, IL-17B did not affect the melanoma cell line A375, which expresses low levels of IL-17RB (Figure 2a). The two ligands of the IL-17RB receptor may mediate distinct mechanisms, with IL-17B and IL-17E exhibiting different functions (Reynolds et al. 2015). This finding is particularly relevant for IL-17E, which has been shown to promote the proliferation of HCC cells (Luo et al. 2016). The use of an IL-17RB-blocking peptide prevented the inhibitory effects of IL-17B on HCC cells, confirming that the observed effects are mediated through the IL-17RB receptor (Figure 2b). As expected, heat-inactivated IL-17B was unable to inhibit the proliferation of HepG2 cells, as shown in Figure 2c. To confirm the effect of IL-17B on the proliferation of selected cell lines, we performed a colony formation assay with HepG2 and A375 cells treated with 750 ng/mL of IL-17B. Consistent with the BrdU assay results, we observed no effect of IL-17B on colony formation in the melanoma cell line A375 (Figure 2d). However, in HepG2 cells, IL-17B treatment reduced colony formation by approximately 45% (Figure 2e). Previous studies have suggested that IL-17B may activate NF- κ B signaling (Huang et al. 2014), so we sought to determine

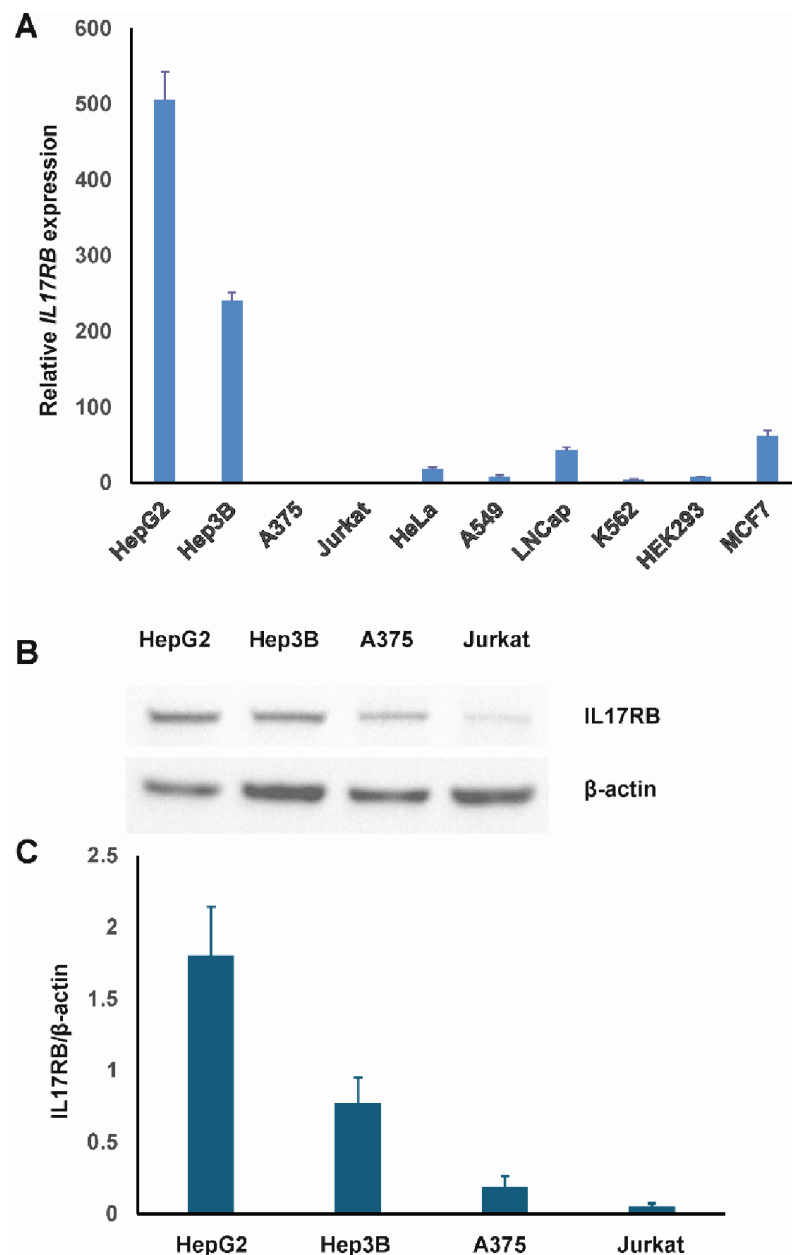


Fig 1. Expression of human IL-17RB in cells of different origins. **(A)** mRNA levels of IL-17RB in selected cell lines. The results are presented as the means \pm SDs, $n = 4$. **(B)** Protein levels of IL-17RB in HepG2, Hep3B, A375, and Jurkat cell lines, with β -actin used as a loading control. **(C)** Densitometric analysis of the Western blot bands was performed using ImageJ (<https://imagej.net/ij/>). The results are shown as the means \pm SDs, $n = 3$. SDs, standard deviations.

whether this is also the case in HepG2 cells. To investigate this possibility, we performed Western blot analysis for phosphorylated I κ B α , an inhibitor of NF- κ B that blocks the nuclear localization signals of NF- κ B. Phosphorylation of I κ B α disrupts the complex, leading to NF- κ B activation (Jacobs and Harrison 1998; Mathes et al. 2008). We observed a dose-dependent increase in I κ B α phosphorylation (Figures 3a and 3b), which typically indicates the activation of NF- κ B. However, contrary to expectations (Tan et al. 2020), we did not detect an increase in

reporter activity when an NF- κ B-dependent reporter vector was used, although TNF- α treatment successfully induced reporter activity (Figure 3c). These findings suggest the presence of a mechanism that prevents NF- κ B activation in hepatocellular cells following IL-17B treatment, despite the phosphorylation of I κ B α . This finding implies that IL-17B might not promote NF- κ B activity in HCC cells, a factor associated with hepatocellular carcinogenesis (Czaderna et al. 2019; Tan et al. 2020). However, we cannot exclude the possibility that this HCC cell line already

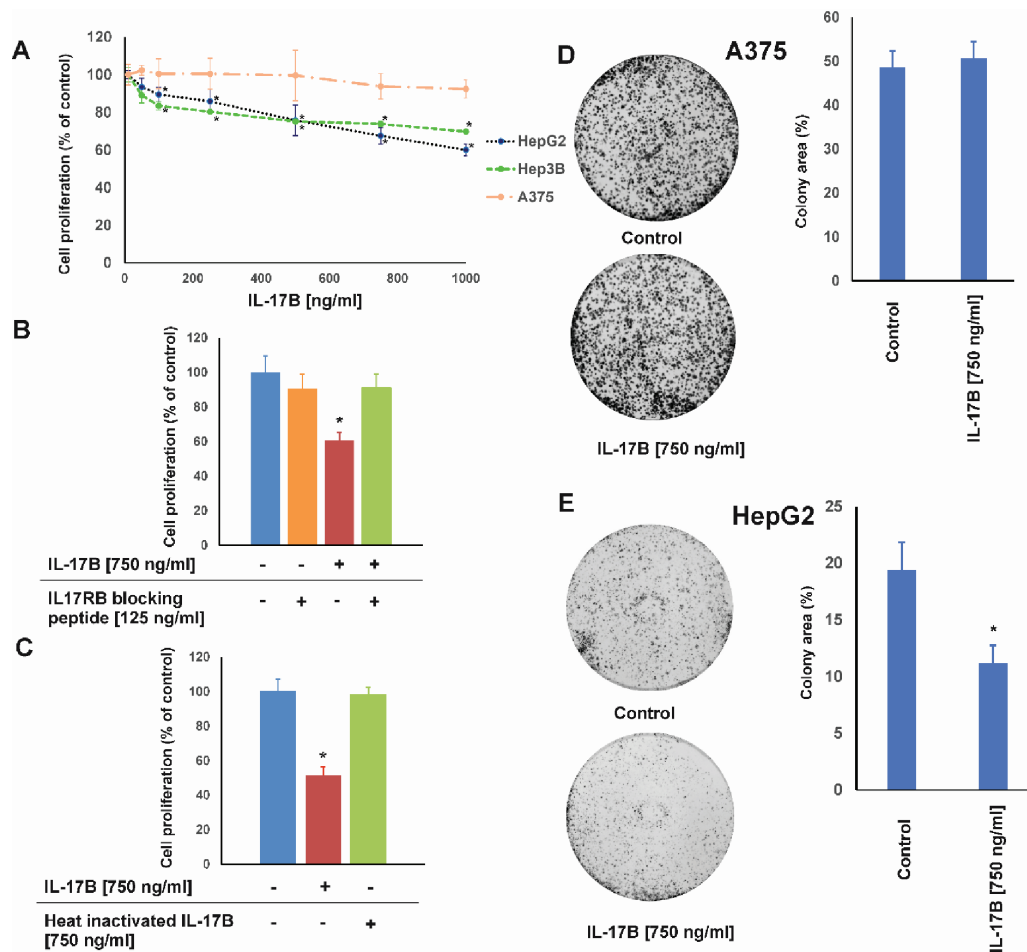


Fig 2. IL-17B inhibits the proliferation of HCC cell lines with high IL-17RB expression. (A) BrdU assay results show the effects of increasing concentrations of IL-17B on the proliferation of HepG2, Hep3B, and A375 cells. The results are presented as the means \pm SDs, $n = 4$, with * indicating statistical significance at $p < 0.05$. (B) The addition of an IL-17RB-blocking peptide inhibited the effects of IL-17B on HepG2 cell proliferation, as determined by the BrdU assay. The data are presented as the means \pm SDs ($n = 6$), with * indicating statistical significance at $p < 0.05$. (C) Effects of native and heat-inactivated IL-17B on HepG2 proliferation. The results are presented as the means \pm SDs, $n = 4$, with * indicating statistical significance at $p < 0.05$. (D) Colony formation assay results show the effect of IL-17B on A375 cells, with colony area values shown in the right panel. The results are presented as the means \pm SDs, $n = 3$. (E) Colony formation assay results show the effect of IL-17B on HepG2 cells, with colony area values displayed in the right panel. The results are presented as the means \pm SDs, $n = 3$, with * indicating statistical significance at $p < 0.05$. HCC, hepatocellular carcinoma; SDs, standard deviations.

exhibits high constitutive expression or activation of NF- κ B, as previously observed in U937 leukemia cells treated with IL-17A (Adunyah et al. 2021). Previous studies have shown that liver cancer is often driven by the activation of the AKT signaling pathway and that glycolytic activity increases during HCC progression (Tian et al. 2023). Therefore, we analyzed AKT phosphorylation in HepG2 cells treated with IL-17B and found that IL-17B treatment reduced AKT phosphorylation (Figures 3d and 3e), suggesting that this signaling pathway may be involved in the observed effects on cell proliferation. To gain a broader understanding of the cellular response, we performed RNA-seq analysis on HepG2 cells treated with IL-17B for 24 h. Our analysis revealed that 241 genes exhibited changes in expression

following cytokine treatment (Figure 4). Among these genes, 74 were associated with antisense RNAs, small nucleolar RNAs, or long non-coding RNAs (data not shown). In accordance with our data, we did not identify genes whose expression is NF- κ B-dependent in HepG2 cells. GO analysis revealed several enriched cellular component terms that reached statistical significance, including the cation channel complex, voltage-gated potassium channel complex, and potassium channel complex (Figure 4c). Additionally, Reactome pathway analysis revealed that IL-17B treatment affected pathways related to potassium channels, selenoamino acid metabolism, NMD, and rRNA processing in the nucleolus and cytosol (Figure 4d). Interestingly, some of these pathways are linked to HCC progression and are

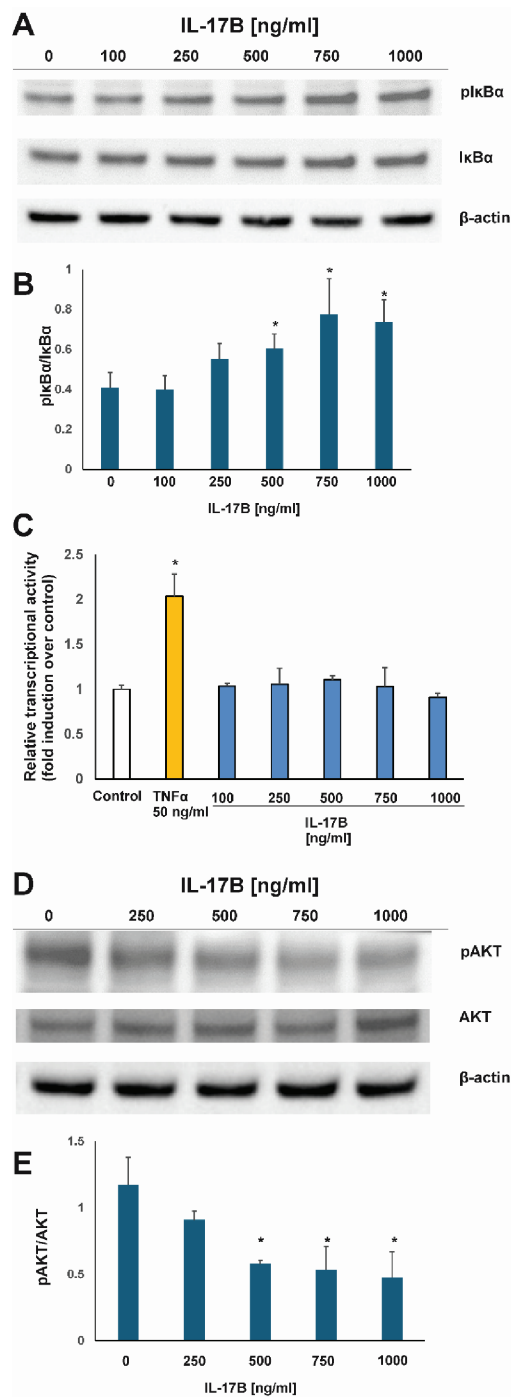


Fig 3. IL-17B induces the phosphorylation of IκBα without activating NF-κB and concurrently inhibits AKT phosphorylation. **(A)** Effects of increasing concentrations of IL-17B on IκBα phosphorylation in HepG2 cells treated with the cytokine for 24 h, as determined by Western blotting. Total IκBα and β-actin levels are also shown. **(B)** Densitometric analysis of the pIκBα/IκBα ratio from three independent experiments was performed using ImageJ. *Indicates statistical significance at $p < 0.05$. **(C)** IL-17B does not trigger NF-κB-dependent transcription in HepG2 cells, as evidenced by transient transfection with an NF-κB reporter plasmid. After seeding, the cells were transfected with the reporter plasmid and pCMV-SEAP (transfection control). Following a 24-h treatment with IL-17B and TNF-α (positive control), the cells were harvested and lysed, and luciferase activity was measured. The results were normalized to Secreted Alkaline Phosphatase (SEAP) activity and are presented as the means \pm SDs ($n = 6$). *Indicates statistical significance at $p < 0.05$. **(D)** Effects of increasing concentrations of IL-17B on AKT phosphorylation in HepG2 cells treated with the cytokine for 24 h, as determined by Western blotting. Total AKT and β-actin levels are also shown. **(E)** Densitometric analysis of the pAKT/AKT ratio from three independent experiments was performed using ImageJ. *Indicates statistical significance at $p < 0.05$. SDs, standard deviations; TNF-α, tumor necrosis factor-α.

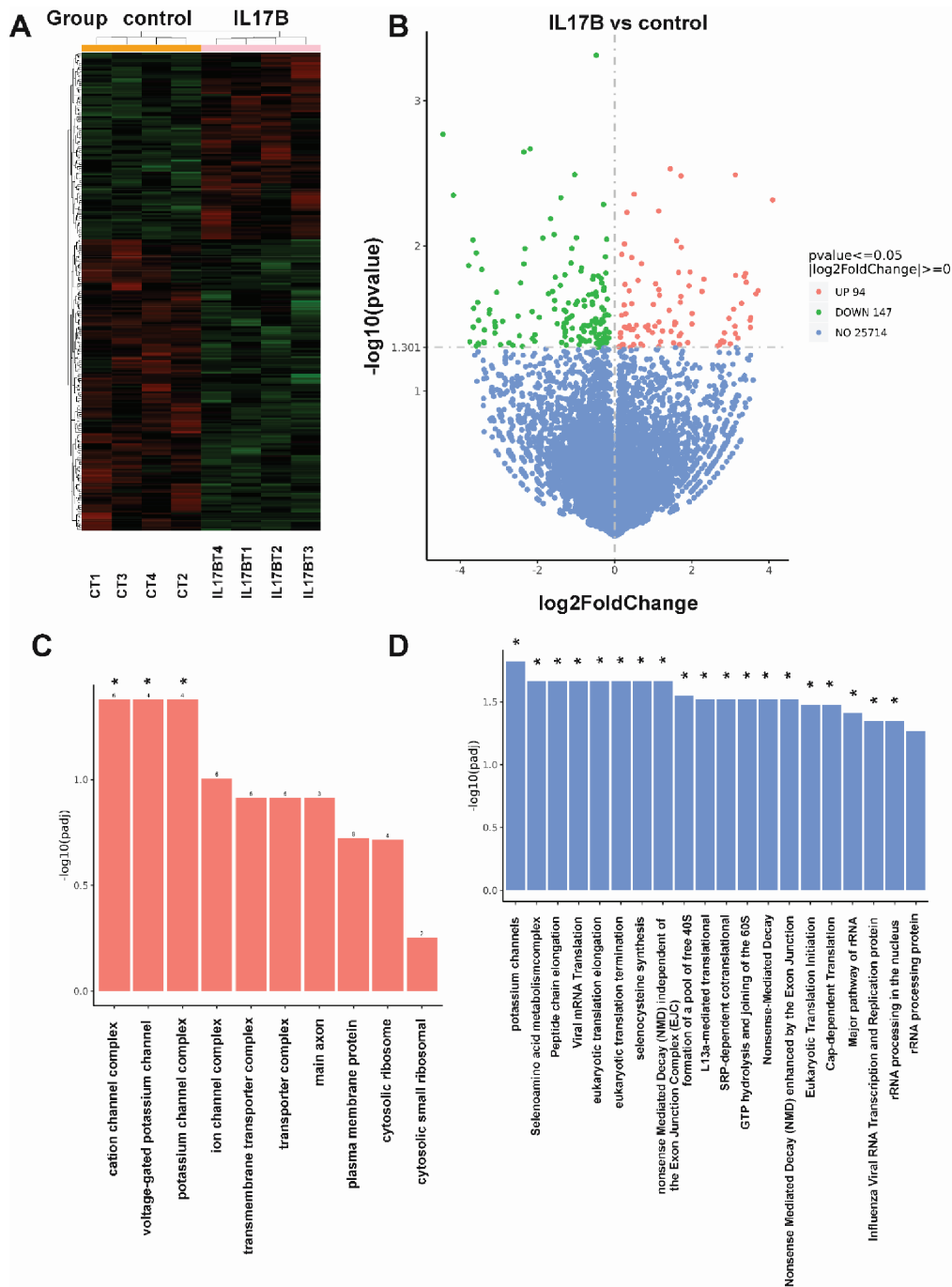


Fig 4. RNA-seq analysis revealed that IL-17B (750 ng/mL) altered the transcriptomic profile of HepG2 cells. **(A)** Hierarchical clustering heatmap displays differentially expressed genes across all the samples. The FPKM cluster analysis results are shown, where each column represents an individual sample (IL-17B-treated vs. control), and each row corresponds to a specific gene. Red indicates high gene expression levels, whereas green signifies low gene expression levels. **(B)** Volcano plot illustrates differentially expressed genes in HepG2 cells treated with 750 ng/mL of IL-17B for 24 h. Genes with a $\log_2(\text{fold change})$ of 1 and a p-adjusted value of 0.05 were considered significantly different. The red dots represent genes whose expression was significantly upregulated, the blue dots indicate genes whose expression was significantly downregulated, and the gray dots represent genes whose expression did not significantly change. **(C)** Results of GO (cellular component) enrichment analysis of significantly differentially expressed genes after IL-17B treatment of HepG2 cells. **(D)** Reactome pathways associated with the differentially expressed genes after IL-17B treatment of HepG2 cells. FPKM, fragments per kilobase of transcript per million mapped reads; GO, gene ontology; RNA-seq, RNA sequencing.

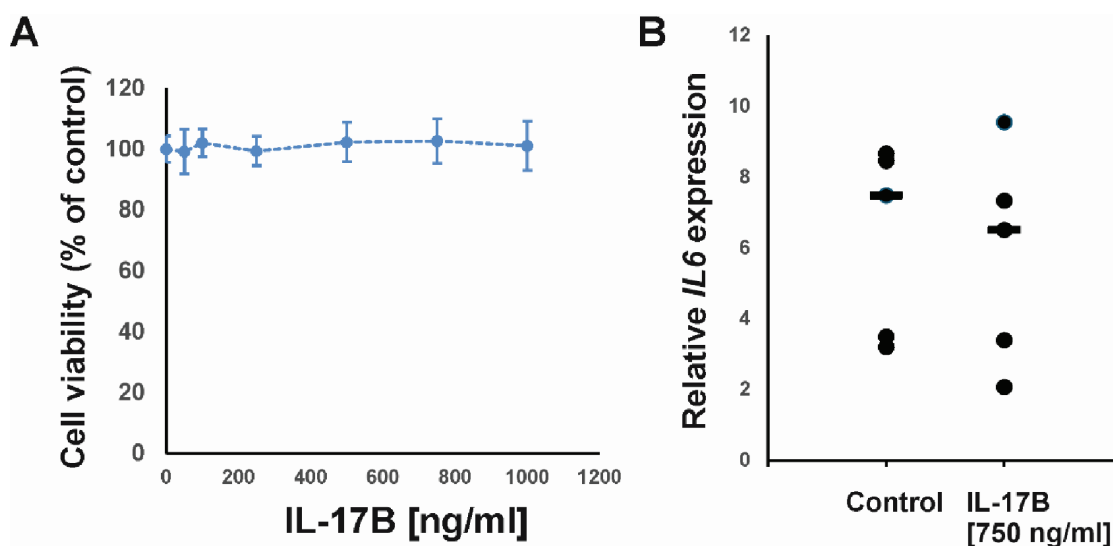


Fig 5. Effects of IL-17B on human primary monocytes. (A) IL-17B does not affect the viability of human primary monocytes, as determined by the CellTiter-Glo® Luminescent Cell Viability Assay (Promega). Human primary monocytes were treated with increasing concentrations of IL-17B for 48 h. After incubation, the cells were lysed, and luminescence was measured. Data are presented as mean \pm SD ($n = 6$). (B) Effect of IL-17B (750 ng/mL) on IL6 expression in human primary monocytes. Cells were treated with IL-17B (750 ng/mL) for 48 h, after which they were collected and subjected to RNA isolation. Data are shown as dot plots with bars indicating the median values from five individual donors. SD, standard deviation.

considered potential targets for anti-HCC therapy, e.g., those associated with potassium channels and NMD (Tan et al. 2022; Chen et al. 2023). Since the effects of IL-17B on HCC cells were observed at relatively high concentrations, it was important to demonstrate that such concentrations do not affect normal blood cells. Therefore, we investigated the impact of IL-17B on the viability of human primary monocytes isolated from PBMCs. As shown in Figure 5a, IL-17B treatment did not reduce monocyte viability. Monocytes are an important source of IL-6, an IL that plays a pivotal role in HCC cell proliferation (Liu et al. 2010; Xu et al. 2021). Therefore, we investigated whether IL-17B induces *IL6* expression, as this would represent a significant contraindication for the use of IL-17B in patients. However, as shown in Figure 5b, IL-17B treatment does not induce *IL6* transcription in monocytes.

Although IL-17B was discovered nearly 25 years ago, it remains an understudied cytokine. Most research has focused on its effects on specific cancer cells, revealing pleiotropic effects that vary by cancer type (Bie et al. 2017a). For example, Huang et al. (2014) reported that the activation of NF- κ B by IL-17B promotes breast tumorigenesis via the activation of antiapoptotic pathways and promotes resistance to paclitaxel (Laprevotte et al. 2017). Wu et al. (2015) reported that elevated IL-17RB expression is closely associated with an increased risk of metastasis and shorter progression-free survival in pancreatic cancer patients. IL-17RB is markedly upregulated in gastric cancer tissues compared with non-cancerous tissues, and its overexpression is linked to poor patient prognosis (Bie et al. 2016). In contrast, IL-17B inhibited the growth of human

endothelial cells, impaired their adhesion to the extracellular matrix, inhibited their migration, and suppressed tubule formation in a Matrigel assay. These findings suggest that IL-17B may possess antiangiogenic properties (Sanders et al. 2010). Wang et al. (2025) reported that IL-17RB expression in colorectal cancer decreases as the tumor stage increases, with higher IL-17RB levels linked to improved patient prognosis, indicating its potential role in disease progression. Additionally, IL-17B expression is positively correlated with CD4⁺ T lymphocyte and mast cell infiltration (Wang et al. 2025). To our knowledge, this is the first study to demonstrate that HCC cells, among other cells, exhibit high IL-17RB expression and that the IL-17RB ligand IL-17B can inhibit their proliferation. Why is this significant? First, the main source of IL-17B in the tumor microenvironment may be neutrophils (Al-Samadi et al. 2016), which exhibit both protumor (N2) and antitumor (N1) properties in HCC (Arvanitakis et al. 2021). Antitumor neutrophils suppress tumor growth and metastasis through both direct cytotoxic effects and the activation of the immune system by stimulating innate and adaptive immune responses, engaging T and B lymphocytes, natural killer cells, and other cells (Jaillon et al. 2013; Arvanitakis et al. 2021). Thus, further exploration of the antitumor potential of neutrophils in HCC and IL-17B release could be highly valuable for HCC patients. Second, HCC patients face progressive liver dysfunction (Sun and Sarna 2008). Ichinohe et al. (2017) reported that the IL-17B–IL-17RB signaling pathway facilitates liver regeneration by stimulating the expansion of endogenous hepatic progenitor

cells. Therefore, enhancing the release of IL-17B or treatment with exogenous IL-17B could protect liver function and support liver recovery in HCC patients. However, further studies are needed to determine whether inhibiting IL-17E–IL-17RB signaling or stimulating IL-17B–IL-17RB provides greater benefit for HCC patients.

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Credit Authorship Contribution Statement

J.P.: Conceptualization, methodology, investigation, manuscript writing, and funding acquisition. I.K.: Conceptualization, methodology, and investigation. K.K., D.G., and A.S.: Methodology and investigation. M.R.: Conceptualization, methodology, investigation, and manuscript writing.

Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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