

Evaluation of TRAM@PPF Nanoparticles for Efficacy Against Pancreatic Cancer in Mice Model

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Introduction: Pancreatic ductal adenocarcinoma (PDAC) poses a major challenge due to the lack of effective treatment options and its extremely poor prognosis. Nanodrug delivery systems can improve drug solubility and enable efficient targeted delivery, offering new possibilities for PDAC therapy.

Methods: The oncogenic role of KCa3.1 in PDAC was validated through analyses of The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) databases combined with functional assays. To overcome the limitations of conventional therapies, we developed a targeted nanodrug delivery system, TRAM@PPF, based on PLGA nanoparticles modified with polyethylene glycol-folate (PEG₂₀₀₀-FA). This system was prepared by the emulsion-solvent evaporation method to specifically deliver the KCa3.1 channel inhibitor TRAM-34 to PDAC cells. We characterized the nanosystem's physical properties and release profile and evaluated its antitumor efficacy in vitro and in vivo.

Results: The synthesized TRAM@PPF nanoparticles demonstrated uniform size (~142 nm) and excellent stability, with superior cellular uptake compared to non-folate-modified nanoparticles. In vitro, TRAM@PPF showed potent antitumor activity by markedly inhibiting cell proliferation and enhancing apoptosis. Following intravenous administration in pancreatic cancer mouse models, TRAM@PPF significantly inhibited tumor growth, reduced tumor weight, and prolonged survival. Moreover, TRAM@PPF showed excellent biosafety in animal models, suggesting strong potential for further clinical translation in PDAC therapy.

Conclusion: TRAM@PPF preserves folate-mediated tumor-targeting capability while significantly enhancing antitumor activity, offering a promising strategy for targeted therapy of pancreatic cancer.

Keywords: nanoparticles, PDAC, KCa3.1 inhibitor

Introduction

Pancreatic cancer, often referred to as the “king of cancers” because of its extremely poor prognosis, is recognized as one of the most lethal tumors within the gastrointestinal system.^{1–3} The predominant form, pancreatic ductal adenocarcinoma (PDAC), is characterized by an exceptionally low 5-year survival rate and swift progression. A critical factor contributing to this poor prognosis is the unique tumor microenvironment (TME) of PDAC, which is defined by a dense, desmoplastic stroma composed of activated fibroblasts and excessive extracellular matrix. This fibrotic barrier not only promotes tumor cell survival but also creates high interstitial fluid pressure that collapses intratumoral blood vessels, significantly impeding the delivery of systemic therapies.^{4–7} Currently, treatment options for PDAC remain limited; most patients undergo standard chemotherapy or radiotherapy, which provide only marginal improvements in survival rates.^{8–10} These considerable clinical obstacles highlight the urgent need for innovative therapeutic approaches that can effectively bypass these stromal barriers and target specific molecular drivers within the PDAC microenvironment.^{11–14}

Among numerous molecular targets, the medium-conductance calcium-activated potassium channel KCa3.1 has emerged as a key driver of tumor progression. Structurally similar to the KCa2 channel, it functions as an oncogenic regulator by maintaining membrane hyperpolarization, providing the essential electrochemical drive for sustained calcium influx. This ionic signaling is crucial for activating downstream pathways that regulate cell cycle progression and uncontrolled proliferation in PDAC cells. Furthermore, KCa3.1 plays a pivotal role in tumor migration and invasion by acting as a “hydrodynamic engine”. By coordinating potassium efflux with water movement, it facilitates the localized volume changes and cytoskeletal remodeling required for cancer cells to navigate through the dense PDAC stroma.^{15–17} Bioinformatic analysis of The Cancer Genome Atlas (TCGA) data, complemented by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses, revealed that elevated KCa3.1 expression correlates with poor clinical outcomes in PDAC. Furthermore, both silencing KCa3.1 genetically and inhibiting it pharmacologically disrupt intercellular communication and reduce tumor cell survival,¹⁸ underscoring the therapeutic potential of targeting KCa3.1 in PDAC.

TRAM-34, a selective inhibitor of the KCa3.1 channel, has demonstrated efficacy in suppressing tumor cell growth and metastasis by blocking channel function.^{19–21} Nevertheless, its clinical translation is hampered by poor water solubility and non-specific distribution in vivo. Advanced nanocarrier systems offer solutions to these challenges by improving drug solubility, extending systemic circulation, and enabling encapsulation-based delivery.^{22–24} Such nanosystems also provide opportunities for ligand-mediated targeting,^{25–28} tumor microenvironment-responsive drug release,^{29–31} and the combination of chemotherapy with targeted therapies.^{32–34} In preclinical PDAC models, nanomedicines have shown enhanced tumor-specific drug delivery and minimized systemic side effects.^{35–37}

To address the delivery limitations of TRAM-34, we designed a PLGA-PEG₂₀₀₀-FA (PPF) nanocarrier, consisting of a PLGA core for biocompatibility and controlled degradation.^{38–40} The PEG₂₀₀₀-FA is strategically employed for its dual functionality: the polyethylene glycol (PEG) moiety prolongs systemic circulation time by providing steric stability,^{41,42} while the folic acid (FA) moiety facilitates precise receptor-mediated targeted delivery through high-affinity binding and efficient cellular uptake, leveraging the significant overexpression of folate receptor (FR) in PDAC cells.³³ Utilizing this multifunctional nanoplatform, we successfully synthesized TRAM-34-loaded PPF nanoparticles (TRAM@PPF) via an optimized emulsion-solvent evaporation technique.^{43,44} We performed comprehensive physicochemical characterization and assessed their therapeutic efficacy in both in vitro and in vivo PDAC models. Our findings indicate that TRAM@PPF effectively targets tumor sites, exhibiting potent antitumor activity and significantly inhibiting PDAC cell proliferation. Therefore, TRAM@PPF establishes a solid foundation for improving the clinical application prospects of TRAM-34.

Materials and Methods

Materials

PLGA, TRAM-34, CH₂Cl₂ (DCM), polyvinyl alcohol (PVA) and PEG₂₀₀₀-FA were purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Cell culture reagents were purchased from Zhongqiao Xinzhou Biotechnology Co., Ltd. (Shanghai, China). CCK-8 Cell Counting Kit, Annexin V-FITC/PI, Anti-GAPDH and anti-KCa3.1 antibodies were acquired from Zeheng Biotechnology Co., Ltd. (Chongqing, China). BUN, ALT, and AST kits were obtained from Jingmei Biotechnology Co., Ltd. (Jiangsu, China).

Differential Expression Analysis

Gene Expression Profiling Interactive Analysis 2 (GEPIA2) was used to analyze the expression of the KCa3.1 gene in various cancer tissues. Combining samples from TCGA and data from Genotype-Tissue Expression (GTEx) project, we further investigated the differential expression of KCa3.1 in cancer and corresponding normal tissues.

Evaluation of Prognostic Features of Pancreatic Ductal Adenocarcinoma

We employed Kaplan-Meier survival analysis to assess the overall survival outcomes of pancreatic cancer patients with high and low KCa3.1 expression in the TCGA cohort. KEGG pathway analysis was also performed on the differentially expressed genes.

Single Cell Analysis of Pancreatic Cancer

The R package (Seurat) was used for PDAC data integration and quality control. Cell types were annotated using marker genes (IGHG2; CD68; COL1A1; SLC4A4; S100A2; CD2; MS4A1; PRSS1; PLVAP; RGS5). The reduced dimensionality was visualized using the UMAP function. The expression levels of KCa3.1 in different cell types were visualized using the Vlnplot and Featureplot functions.

In vitro Cell Experiments

The pancreatic ductal adenocarcinoma cell line PANC-1 was obtained from the American Type Culture Collection. This cell line was cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) at 37°C in a humidified 5% CO₂ atmosphere. The medium was refreshed periodically to maintain optimal cell viability. At the logarithmic growth phase, cells were trypsinized, counted, and seeded into 6-well or 96-well plates for further experiments. After allowing sufficient time for cell attachment, further treatments were performed.

siRNA Transfection for KCa3.1 Knockdown

The small interfering RNA (siRNA) targeting the KCa3.1 gene and the control siRNA were synthesized by GenePharma. The siRNA sequences used in this study are listed in [Table S1](#). The siRNA was transiently transfected using Lipofectamine 3000. Cells were harvested for subsequent experiments 48 hours post-transfection.

Quantitative Real-Time PCR Analysis

Total RNA was extracted from cell precipitates using TRIzol solution. 1 µg of total RNA was then reverse transcribed into cDNA using a HiScript III RT SuperMix for qPCR kit. The resulting cDNA was amplified via qPCR with ChamQ Universal SYBR qPCR Master Mix. GAPDH served as the internal reference gene, and the relative expression of the target gene was calculated using the $2^{-\Delta\Delta C_t}$ method. All primer sequences are detailed in [Table S2](#).

Western Blot Analysis

Cell pellets were lysed on ice using RIPA buffer. Anti-KCa3.1 and anti-GAPDH antibodies (both at a 1:1000 dilution ratio) were used to probe for target proteins. HRP-conjugated goat anti-rabbit secondary antibody was applied, and bands were visualized with ECL chemiluminescence reagent.

Cell Proliferation Assay

Cell viability was measured using the CCK-8 assay. Cells were seeded in 96-well plates, assigned to either control or KCa3.1 knockout groups, and incubated. At 0, 24, and 48 hours post-treatment, 10 µL of CCK-8 reagent was added to each well and incubated for 1 hour at 37°C. Absorbance at 450 nm was recorded with a microplate reader, and cell viability was determined from the optical density values.

The Preparation of TRAM@PPF Nanoparticles

We prepared TRAM@PPF using an improved emulsion-evaporation method.^{45,46} To prepare TRAM@PPF, PLGA (20 mg) and TRAM-34 (2 mg) were dissolved in 5 mL dichloromethane (DCM) and sonicated for dispersion. This organic phase was then added dropwise into 20 mL of a 3% (w/v) PVA solution containing PEG₂₀₀₀-FA (10 mg), maintaining an organic-to-aqueous phase ratio of 1:4. The mixture was placed in an ice bath and subjected to ultrasonication at 100 W for 5 minutes to form a stable oil-in-water (O/W) emulsion. After forming the O/W emulsion, the DCM was removed by evaporation using a rotary evaporator at 25 °C under reduced pressure for 4 h. This temperature was selected to ensure the efficient removal of the organic solvent while maintaining the stability of the loaded TRAM-34. Subsequently, the nanoparticles were collected by centrifugation at 15,000 rpm for 20 minutes at 4°C and washed three times with distilled water. PPF and TRAM@PLGA nanoparticles were prepared similarly and stored at 4°C for subsequent use.

Characterizations of TRAM@PPF Nanoparticles

The morphology of TRAM@PPF was observed using Transmission Electron Microscopy (TEM) (JEM-2100 microscope). Particle size and its change over 12 hours were measured by Dynamic Light Scattering (DLS) performed on Zetasizer Nano Malvern Instruments. The release of TRAM from TRAM@PPF was quantified at various time points in simulated tumor (pH 6.5) and normal cell (pH 7.4) microenvironments.

Cellular Uptake

The TRAM@PPF-Cy5.5 and the TRAM@PLGA-Cy5.5 were prepared by mixing TRAM@PPF and TRAM@PLGA with Cy5.5 solution for 2 hours, followed by dialysis. PANC-1 cells were then seeded into culture dishes and incubated with the synthesized TRAM@PPF-Cy5.5 and TRAM@PLGA-Cy5.5. Cellular uptake was observed after 3 hours of incubation.

In vivo Antitumor Efficacy

The BALB/c nude mice were purchased from Jiangsu Huachuang Xinnuo Pharmaceutical Technology Co., Ltd. Animal experiment license number: SCXK(Su) 2020-0009. All experimental procedures were in accordance with the Chongqing University Guidelines for the Ethical Review of Laboratory Animal Welfare. The PANC-1 cells (2×10^6 in 100 μ L PBS) were subcutaneously injected into the axillae of 4-week-old male nude mice, which were then randomized into four groups. Upon tumor volumes reaching 50–80 mm³, mice received intravenous injections of PBS, PPF, TRAM-34, or TRAM@PPF (5 mg/kg) every two days. Body weight and tumor size were measured, and tumor volumes were calculated as $V = \text{length} \times \text{width}^2 / 2$. At the endpoint, mice were euthanized with 100% CO₂, and tumors along with major organs (heart, liver, spleen, lung, kidney) were collected and weighed. Tumors were fixed in 4% paraformaldehyde, paraffin-embedded, sectioned, and subjected to H&E staining for histopathological evaluation and necrosis. Apoptosis was assessed by TUNEL assay, and caspase-3 expression was quantified via immunofluorescence.

Safety Evaluation

For histopathological assessment, collected organs were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and subsequently stained with hematoxylin and eosin (H&E). The prepared tissue slices were then examined under a light microscope to identify any evidence of inflammation, necrosis, or additional pathological alterations.

Fresh animal blood was utilized for the hemolysis assay. Erythrocyte suspensions were prepared with or without TRAM@PPF at concentrations of 0, 60, 80, 100, and 200 μ g/mL and incubated at 37 °C for 4 hours. After incubation, the supernatant was collected and its absorbance was measured at 570 nm. The percentage of hemolysis was calculated according to the following equation:

$$\text{Hemolysis (\%)} = \frac{\text{OD}_{570\text{TRAM@PPF}} - \text{OD}_{570\text{PBS}}}{\text{OD}_{570\text{UPW}} - \text{OD}_{570\text{PBS}}} \times 100\%$$

For biochemical assessments, peripheral blood was drawn and centrifuged to separate the serum. Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and blood urea nitrogen (BUN) were quantified using an automated biochemical analyzer to evaluate liver and kidney function.

Statistical Analysis

All experimental data were analyzed and plotted using Origin software. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. Quantitative results are presented as the mean $\pm$ standard deviation (SD). Statistical significance was considered at $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

Results and Discussion

KCa3.1 is Aberrantly Overexpressed in Pancreatic Ductal Adenocarcinoma and Correlates with Poor Prognosis

Analysis of the TCGA and GTEx databases revealed that the KCNN4 gene is significantly overexpressed in several types of tumors compared to normal tissue, including CESC; COAD; DLBC; ESCA; LUAD; PAAD, etc. (Figure 1A). In pancreatic cancer, KCa3.1 expression is much higher in tumor tissues than in adjacent non-cancerous tissues (Figure 1B and C). Kaplan-Meier survival curve analysis and Cox analyses indicated that high KCa3.1 expression in pancreatic cancer is associated with shorter overall and progression-free survival (Figure 1D and E), and is linked to poor prognosis. The ROC curve (Figure 1F) shows an area under the curve > 0.5 , indicating good predictive value, sensitivity, and specificity. Cox regression confirmed KCa3.1 as an independent prognostic factor (Figure S1A and Figure S1B), and Figure S1C depicts the correlations between KCa3.1 expression and clinical characteristics including age, gender, tumor grade, and disease status. Collectively, our findings demonstrate that KCa3.1 is significantly overexpressed in pancreatic cancer tissues, highlighting its potential as a prognostic marker for poor outcomes in pancreatic cancer patients.

Expression and Function of KCa3.1 in Pancreatic Cancer Cells and the Tumor Microenvironment

Pancreatic cancer patients from the TCGA database were stratified into high and low KCa3.1 expression groups. Correlation analysis revealed that high KCa3.1 expression was associated with both positively and negatively correlated genes (Figure S2), and these genes were enriched in multiple biological pathways (Figure S3). Immune infiltration analysis showed that the high KCa3.1 expression group exhibited increased levels of stromal and immune cells, particularly memory B cells and Treg cells (Figure S4). Single-cell sequencing further demonstrated that KCa3.1 was highly expressed in malignant pancreatic cancer cells (Figure S5 and S6). The qPCR results demonstrated a significant upregulation of KCa3.1 in PANC-1 cells (Figure 2A). Similarly, it was also found to be significantly upregulated in PANC-1 cells at the protein level (Figure 2B). To investigate the functional role of KCa3.1 in pancreatic cancer, we knocked down KCa3.1 in PANC-1 cells using siRNA. Among the two siRNAs designed, si-KCa3.1-2 showed higher knockdown efficiency than si-KCa3.1-1 (Figure 2C and D), and thus was used for subsequent experiments. The cell viability experiment demonstrated that knockdown of KCa3.1 significantly reduced PANC-1 cell proliferation (Figure 2E) and increased the rate of apoptosis, particularly at early stages (Figure 2F and G). This suggests that KCa3.1 not only contributes to the malignant phenotype of pancreatic cancer cells, but may also influence tumor progression through its effects on the tumor microenvironment.

Characterization, Cellular Uptake, and Apoptosis Induction of TRAM@PPF Nanoparticles

TRAM@PPF was prepared using the emulsion-evaporation method, as illustrated in Scheme 1. Successful synthesis of TRAM@PPF was confirmed by TEM imaging and DLS, revealing uniform spherical morphology with an average diameter of approximately 142 nm and a narrow size distribution (Figure 3A and B). The PDI of the TRAM@PPF is 0.17, with a Zeta potential of -16.3 mV (Figure S7). This favorable size profile is advantageous for biodistribution and passive accumulation within tumors. The Drug Loading Capacity (DLC) of TRAM@PPF was determined to be $6.1\% \pm 0.4\%$, and the Encapsulation Efficiency (EE) was $72.6\% \pm 2.3\%$, measured by UV-visible spectrophotometry. Simultaneously, TRAM@PPF exhibited minimal change in particle size over 12 hours (Figure 3C), indicating its good stability. We evaluated the release behavior of TRAM from TRAM@PPF when incubated at pH 6.5, simulating the acidic microenvironment of solid tumors. Results showed that after 12 hours of incubation, the TRAM release was 60.3% at pH 6.5 and 32.8% at pH 7.4 (Figure 3D). This enhanced release is likely due to the accelerated hydrolysis of the PLGA backbone under acidic conditions, leading to greater TRAM liberation. Modifying nanoparticles with folate (FA) to target tumor cells is a rational strategy for enhancing their cellular uptake.⁴¹ Cellular fluorescence imaging confirmed that PEG₂₀₀₀-FA-functionalized TRAM@PPF achieved

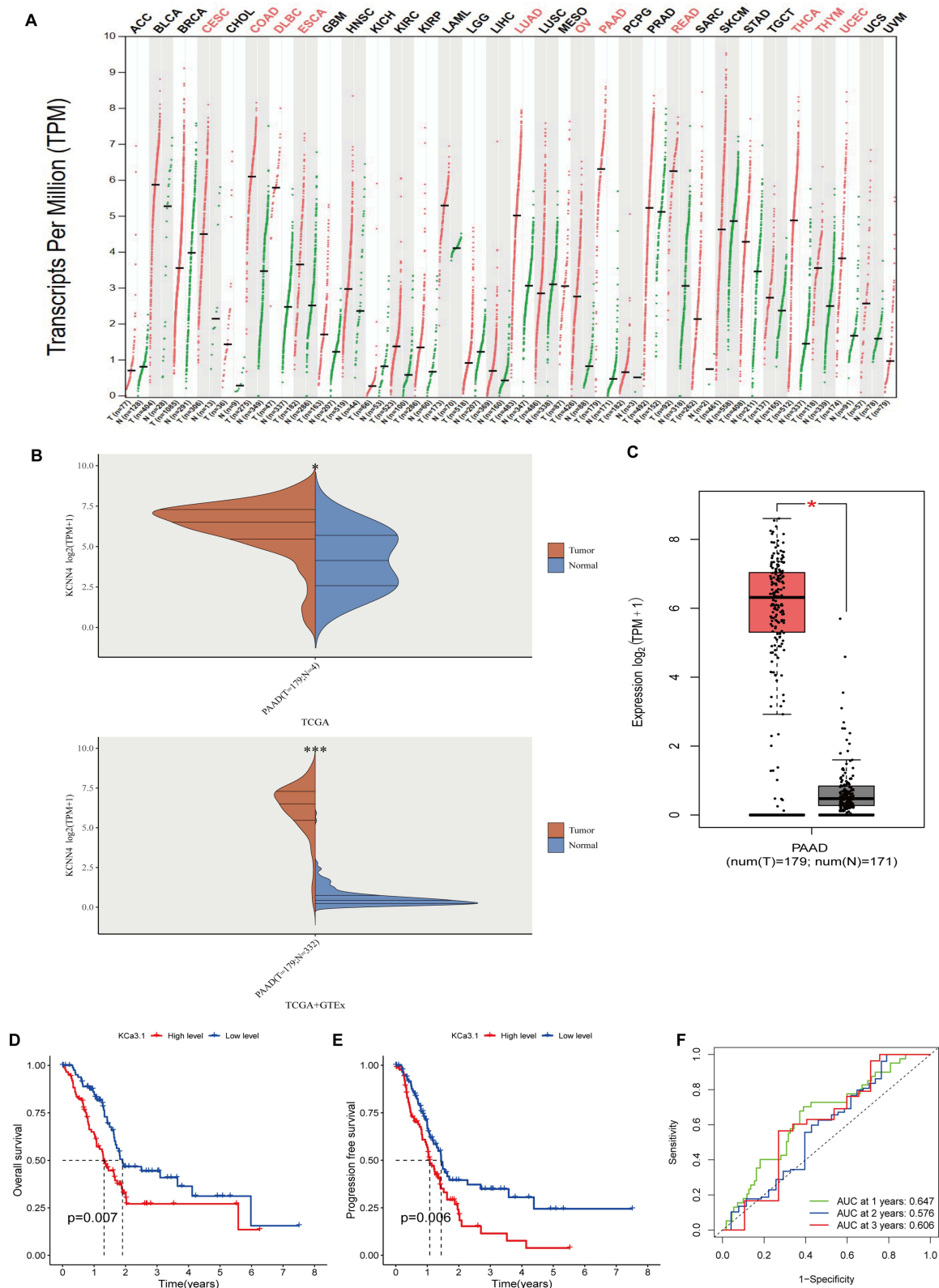


Figure 1 Differential expression and clinical significance of KCa3.1 in cancer. **(A)** KCa3.1 expression in pan-cancer versus normal tissues (TCGA). **(B and C)** KCa3.1 expression in pancreatic cancer versus normal pancreas. **(D and E)** Association of KCa3.1 with overall survival and progression-free survival in PDAC. **(F)** ROC curve for survival prediction in PDAC. * $p < 0.05$.

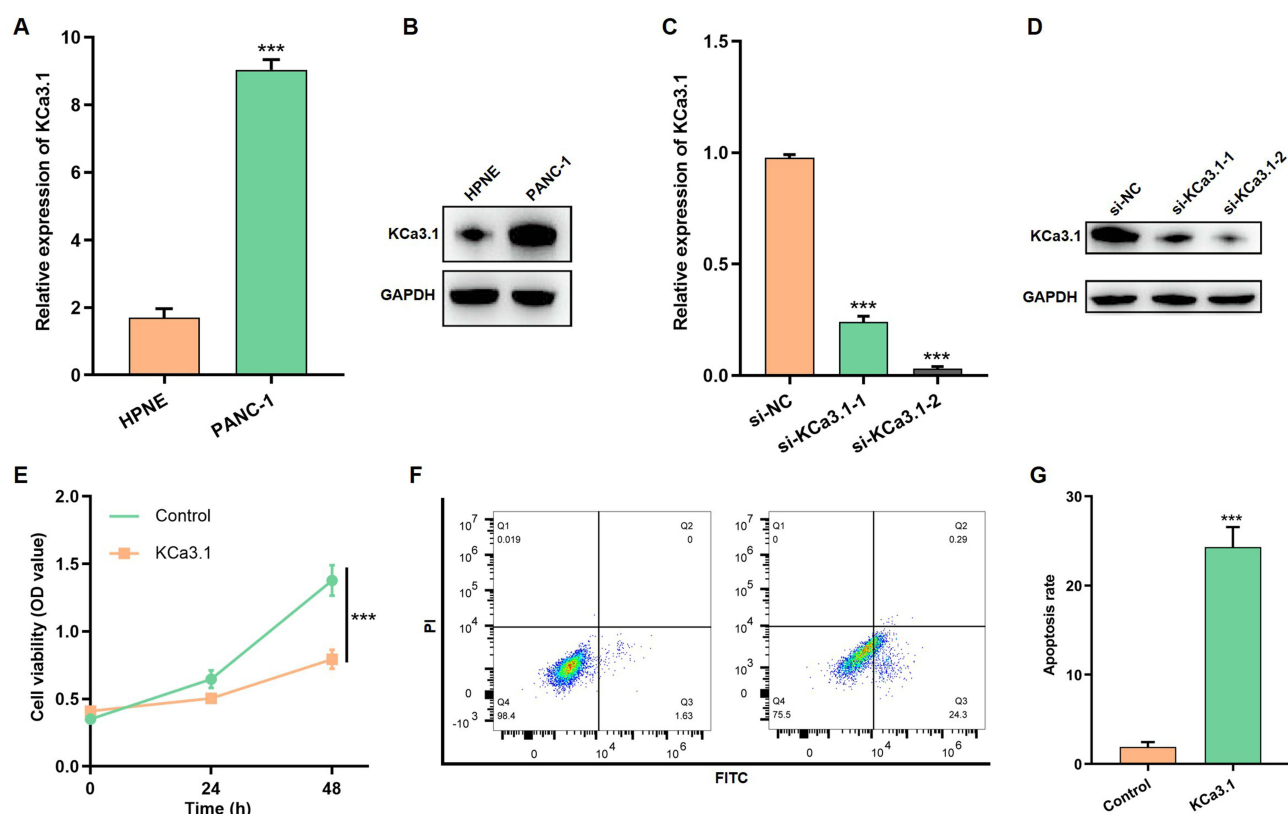
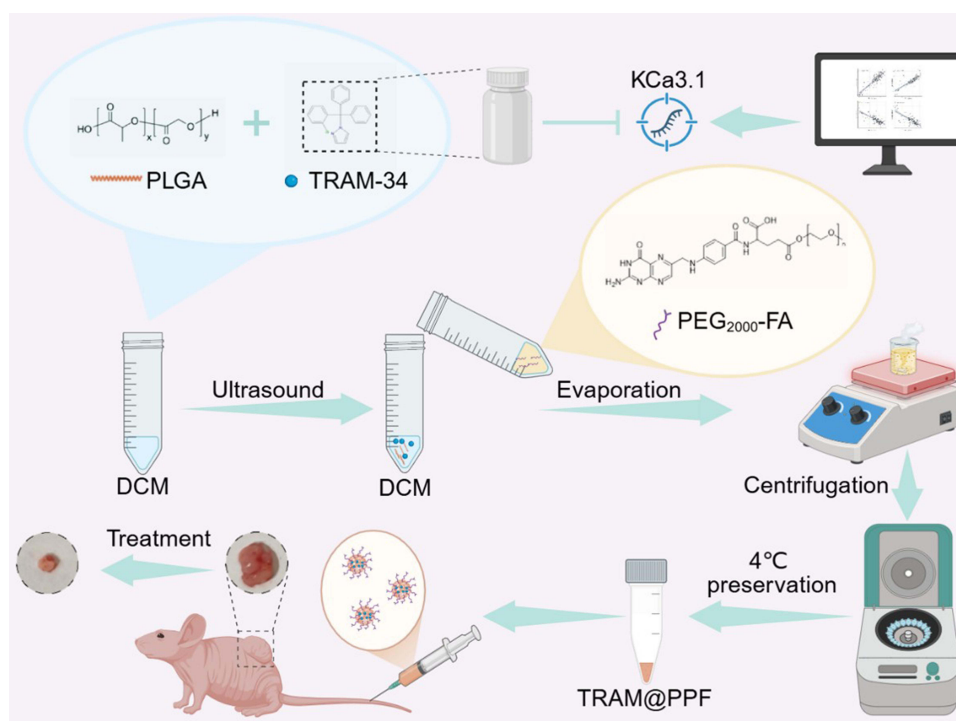


Figure 2 Function of KCa3.1 in pancreatic cancer cells. **(A)** Gene level difference of KCa3.1 between normal pancreatic cells HPNE and pancreatic cancer cells PANC-1. **(B)** Protein level difference of KCa3.1 between normal pancreatic cells HPNE and pancreatic cancer cells PANC-1. **(C)** Knockdown gene level of KCa3.1 in pancreatic cancer cells PANC-1. **(D)** Knockdown protein level of KCa3.1 in pancreatic cancer cells PANC-1. **(E)** Cell proliferation ability of pancreatic cancer cells PANC-1 after knocking down KCa3.1. **(F and G)** Cell apoptosis of pancreatic cancer cells PANC-1 after knocking down KCa3.1. Statistical significance is indicated as *** $p < 0.001$.



Scheme 1 Schematic Illustration of TRAM@PPF Synthesis and PDAC Therapy.

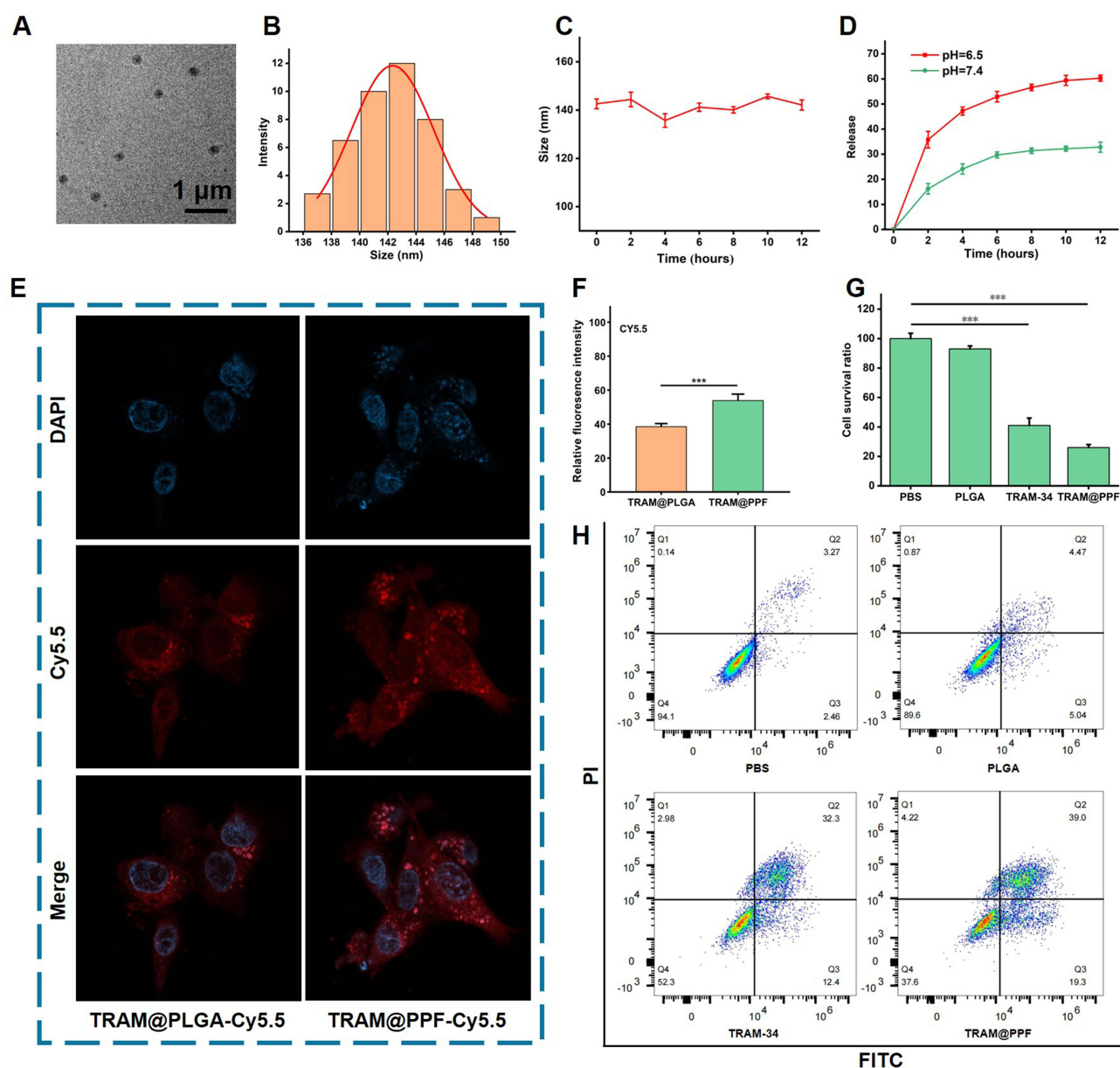


Figure 3 Characterization of the TRAM@PPF. (A) TEM image of the nanoparticles. (B) The size distribution of TRAM@PPF. (C) Stability profile of nanoparticle size over time. (D) Drug release curves showing cumulative drug release from nanoparticles under different pH conditions over time. (E) Fluorescence microscopy images of cellular uptake. (F) Bar graph of quantitative analysis of cellular uptake (mean $\pm$ SD, $n = 3$). (G) Cell viability of PANC-1 cells (mean $\pm$ SD, $n = 3$). (H) Flow cytometry scatter plots of apoptosis in treated cells. Statistical significance is indicated as *** $p < 0.001$.

significantly higher tumor cell uptake, with a 12% increase compared to TRAM@PLGA (Figure 3E and F), consistent with findings reported by Zhang et al for FA-modified nanoparticles in gastric cancer models.⁴³ To further validate the specific localization of TRAM@PPF to folate receptors, PANC-1 cells were pre-incubated with free folic acid to saturate the receptors, followed by treatment with TRAM@PPF (Figure S8). Results demonstrated that the uptake of TRAM@PPF decreased in the presence of free FA. This competitive inhibition confirms that the internalization process of TRAM@PPF primarily depends on folate receptor-mediated mechanisms. Cytotoxicity assays showed that TRAM@PPF induced stronger inhibition of cell viability across all tested concentrations, with particularly pronounced effects at 80 $\mu\text{g/mL}$, where viability was significantly reduced relative to the TRAM-34 control group (Figure S9). Moreover, TRAM@PPF markedly enhanced apoptosis, as evidenced by decreased cell viability (Figure 3G) and an elevated proportion of apoptotic cells detected by flow cytometry (Figure 3H and S10).

To demonstrate the potential broad applicability of TRAM@PPF, we conducted additional CCK-8 assays using Capan-1 cells. As shown in [Figure S11](#), TRAM@PPF exhibited potent inhibitory effects on these cells, reinforcing the therapeutic potential of our nanosystem across different PDAC models. These results demonstrate that TRAM@PPF exhibits potent anti-tumor activity at the cellular level, supporting its further evaluation in vivo studies and potential clinical application.

In vivo Antitumor Activity of TRAM@PPF Nanoparticles

To further evaluate the therapeutic efficacy of TRAM@PPF, its antitumor activity was investigated in a PDAC xenograft model. PANC-1 cells were subcutaneously implanted on day -7, and treatment commenced on day 0. TRAM@PPF was administered via tail vein injection every 48 hours for a total of 14 days, and final tissue samples were collected on day 21 ([Figure 4A](#)). Serial tumor volume assessments revealed that TRAM@PPF significantly suppressed tumor growth compared to controls ([Figure 4B–F](#)). Consistent with these findings, terminal tumor weight analysis showed significantly reduced tumor mass in the TRAM@PPF-treated group relative to controls. Although the use of TRAM-34 alone showed a certain inhibitory effect on tumor growth, but its efficacy is inferior to that of TRAM@PPF, which may be related to its poor tumor-targeting ability ([Figure 4G](#)). Body weight monitoring indicated no significant differences among groups, suggesting minimal systemic toxicity ([Figure 4H](#)). Survival analysis demonstrated that TRAM@PPF markedly prolonged median survival, leading to a cure in 2 out of 6 mice ([Figure 4I](#)). These outcomes can be attributed to two key factors: first, the enhanced permeability and retention (EPR) effect characteristic of tumor vasculature, and second, the active targeting mediated by folate receptor endocytosis. The combination of these mechanisms likely contributed to the observed tumor accumulation and sustained drug release. Histopathological examination revealed lower tumor cellularity by H&E staining, increased TUNEL-positive cells. As indicated by the red arrows in the figure, the caspase-3 immunofluorescence intensity in the TRAM@PPF group was significantly elevated compared to the control group ([Figure 4J](#)). These results indicate that TRAM@PPF exerts pronounced antitumor effects in vivo by inhibiting tumor growth and promoting apoptosis. The superior antitumor performance of TRAM@PPF over free TRAM-34 highlights the critical role of the folate-modified nanocarrier. While free TRAM-34 suffers from rapid clearance and poor solubility, the PPF system leverages the EPR effect and active receptor-mediated endocytosis to bypass the dense desmoplastic stroma of PDAC, which is often a major hurdle for conventional therapies.

Biosafety Assessment of TRAM@PPF Nanoparticles

Following confirmation of the antitumor efficacy of TRAM@PPF, we proceeded to assess the potential biotoxicity of this drug delivery system. Analysis of organ weights demonstrated no significant differences in the heart, liver, spleen, lung, or kidney between the TRAM@PPF treatment group and the control group on day 21, indicating the absence of treatment-induced organ toxicity ([Figure 5A–E](#)). In vitro hemolysis assays showed that TRAM@PPF at a concentration of 100 µg/mL induced a hemolysis rate of 4.7%, remaining below the 5% safety threshold. Further dilution of TRAM@PPF decreased the hemolysis rate, confirming its excellent blood biocompatibility ([Figure 5F](#)). Serum biochemical analysis revealed no significant alterations in key biomarkers compared to PBS controls, with aspartate aminotransferase (AST), alanine aminotransferase (ALT), and blood urea nitrogen (BUN) levels remaining within physiological ranges. This suggests no apparent organ toxicity or pathological changes in mice during the treatment period ([Figure 5G–I](#)). H&E staining of major organs showed normal myocardial structure, orderly hepatocyte arrangement, intact splenic architecture, unobstructed alveolar spaces, and preserved renal morphology, with no evidence of tissue damage ([Figure 5J](#)). These results indicate that TRAM@PPF does not induce detectable systemic toxicity, supporting its potential for safe in vivo application. These results indicate that TRAM@PPF exhibits favorable blood compatibility and no detectable organ toxicity, establishing a fundamental safety basis for future human studies.

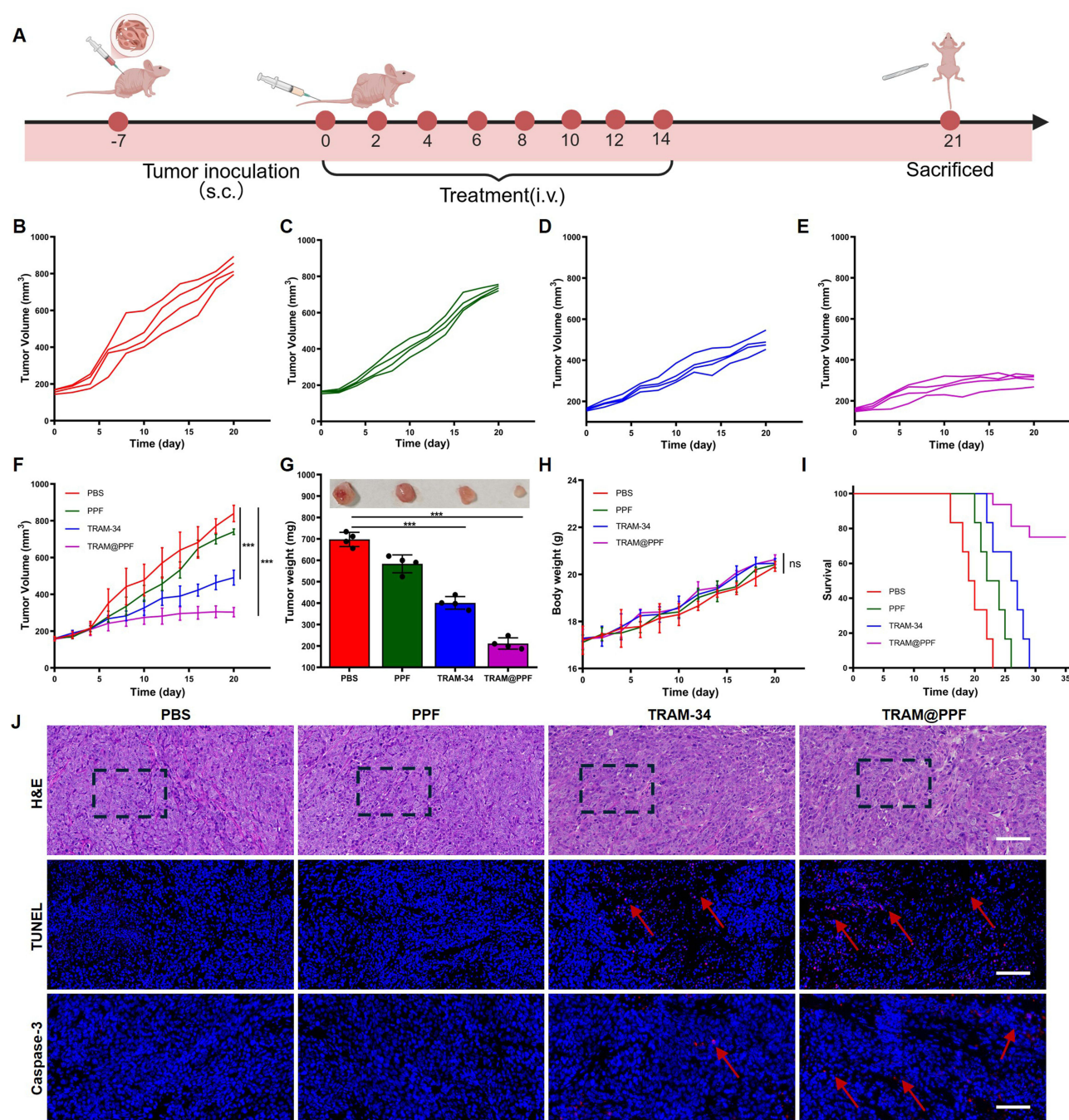


Figure 4 In vivo antitumor efficacy. **(A)** Treatment regimen. **(B)** Tumor volume changes following PBS administration. **(C)** Tumor volume changes following PPF treatment. **(D)** Tumor volume changes following TRAM-34 treatment. **(E)** Tumor volume changes following TRAM@PPF treatment. **(F)** Comparative tumor volume changes across treatment groups. ($n = 4$ per group) **(G)** Tumor weight and representative images of tumors from each group. **(H)** Body weight changes during therapy. **(I)** Survival curves of PANC-1 tumor-bearing nude mice under different treatments. **(J)** Histopathological evaluation of excised tumors. Statistical significance is indicated as *** $p < 0.001$, scale bar = 50 μ m.

Conclusion

In summary, this study combined bioinformatics analysis with experimental validation to identify KCa3.1 as a promising therapeutic target for PDAC. Our bioinformatics analysis and experimental data collectively justify that KCa3.1 is significantly overexpressed in PDAC and correlates with poor patient prognosis, while its knockdown suppresses tumor cell proliferation and induces apoptosis. To overcome the poor solubility and limited tumor-targeting properties of the KCa3.1 inhibitor TRAM-34 in clinical settings, we developed a folate-modified nanomedicine, TRAM@PPF. Nanocarrier encapsulation significantly enhanced

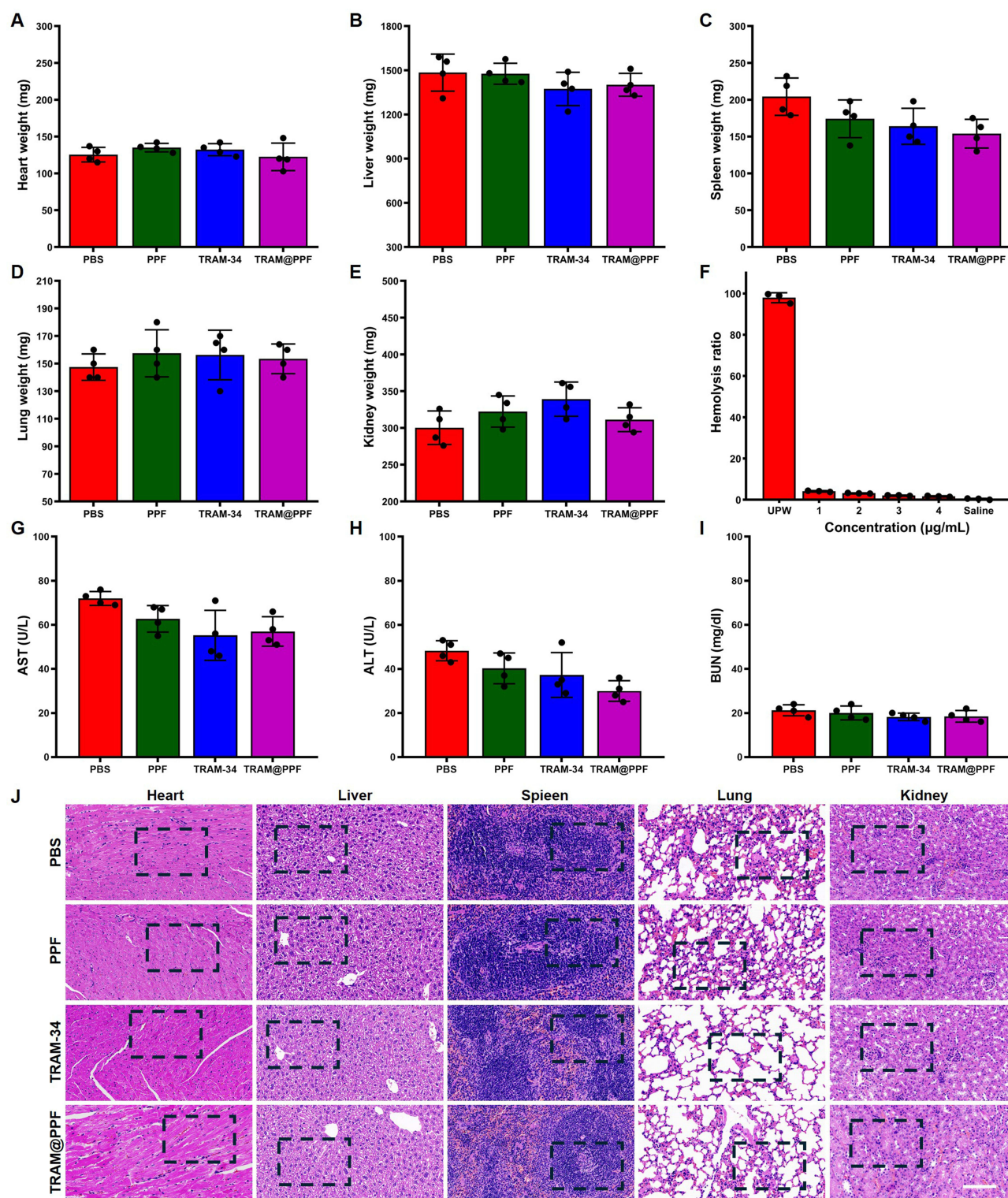


Figure 5 Biosafety evaluation of TRAM@PPF in vivo. (A) Analysis of heart weight, (B) liver weight, (C) spleen weight, (D) lung weight, and (E) kidney weight at the end of the study. (F) Hemolysis rates of TRAM@PPF at various concentrations. (G) Blood biochemical examination: AST levels. (H) Blood biochemical examination: ALT levels. (I) Blood biochemical examination: BUN levels. (J) Representative images of major organs H&E staining. The black dashed box represents a typical image, scale bar = 50μm.

the aqueous solubility of TRAM-34 and facilitated selective delivery to tumor cells, thereby increasing therapeutic efficacy. The superior antitumor activity of TRAM@PPF compared to free TRAM-34 stems from the synergistic effects of passive accumulation and active folate receptor-mediated targeting. Despite its promise as a targeted therapy for pancreatic cancer, challenges remain for clinical translation, including ensuring manufacturing consistency on a large scale, conducting comprehensive long-term safety assessments, and validating clinical efficacy. With continued progress in materials science and nanotechnology, TRAM@PPF holds potential for further clinical optimization through structural refinement, functional upgrades, and combination therapeutic strategies, ultimately aiming to provide pancreatic cancer patients with more effective and safer treatment options.

Ethics Statement

All animal experiments were conducted in accordance with the approval of the Ethics Committee of Chongqing University, approval number A0000202036. All the animal experiments were conducted in compliance with the Regulations for the Administration of Affairs Concerning Experimental Animals of China.

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Disclosure

The authors declare that they have no competing interests in this work.

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