

Metabolomic and Proteomic Analyses Unveil That Polyethylene Glycol–Polycaprolactone-Loaded Curcumin Nanoparticles Induce Mitochondrial Dysfunction and Metabolic Reprogramming to Suppress Melanoma Growth

Yixun Zhang,[†] Xin Zhou,[†] Wenlian Zheng,[†] Jianwei Chen,[†] Qianfeng Xu, Zhenguo Liang, Hongli Zhang, Jundong Lin, Wenjie Xie, Yangjia Zhuo, Muqi Chen, Fen Zou, Biyan Wen, Zeheng Tan, Weide Zhong,^{*} Huikang Yang,^{*} and Ronghua Yang^{*}



Cite This: *ACS Appl. Mater. Interfaces* 2025, 17, 33422–33438



Read Online

ACCESS |



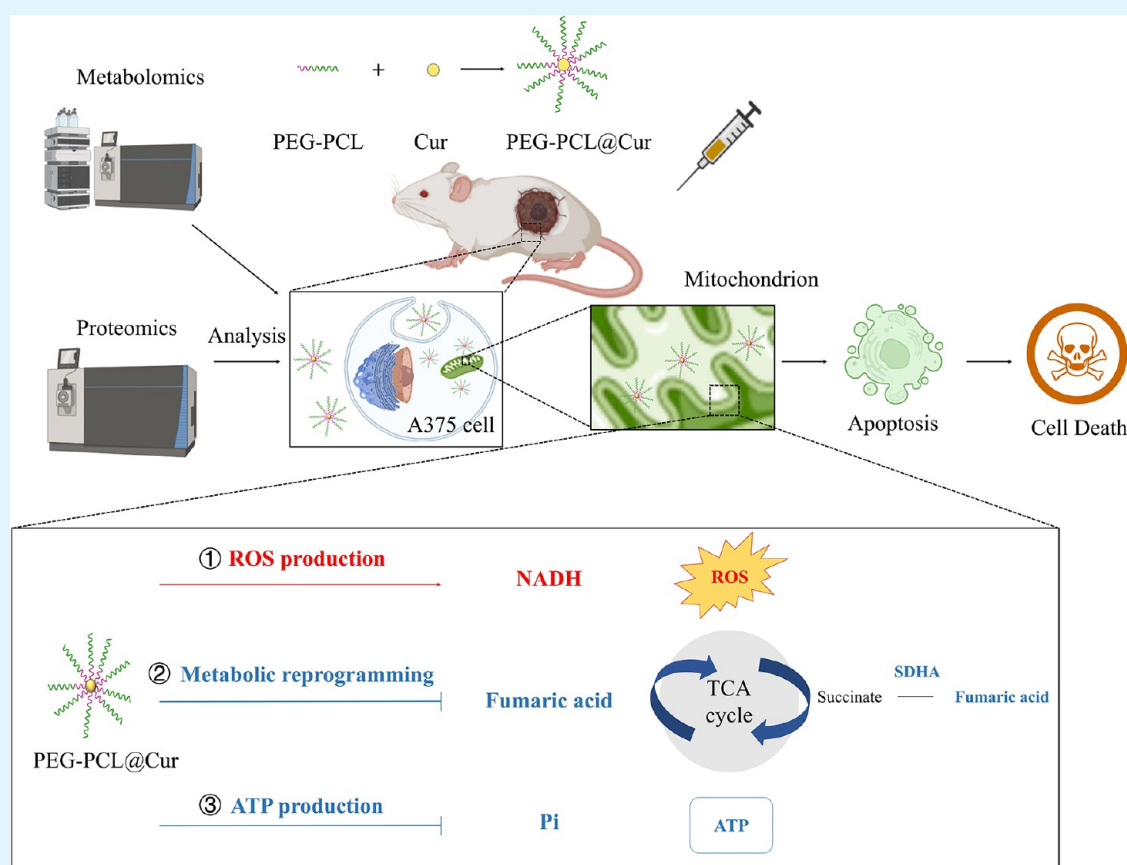
Metrics & More



Article Recommendations



Supporting Information



ABSTRACT: Melanoma is highly dependent on cellular metabolism, but current treatments show limited effectiveness. Despite the promising antitumor properties of curcumin, its clinical utility is limited by its poor bioavailability and potential systemic toxicity. To
continued...

Received: February 11, 2025

Revised: March 25, 2025

Accepted: April 23, 2025

Published: May 27, 2025



address these challenges, we formulated curcumin-loaded polyethylene glycol–polycaprolactone (PEG–PCL) nanoparticles, denoted as PEG–PCL@Cur. We employed omics technologies to investigate their impact on melanoma at the molecular level. In vitro studies revealed that PEG–PCL@Cur notably suppressed the growth, movement, and invasiveness of A375 melanoma cells in a dose-dependent manner. Untargeted metabolomics identified 347 metabolites that were significantly altered. A decrease in fumaric acid levels indicated the suppression of oxidative phosphorylation and a shift in metabolism. Proteomics further confirmed the downregulation of key mitochondrial proteins, such as SDHA, pointing to mitochondrial dysfunction. Mechanistically, PEG–PCL@Cur increased reactive oxygen species (ROS) production by raising NADH levels and inhibiting SDHA expression. This led to a reduction in the level of fumaric acid production and a disruption of the tricarboxylic acid (TCA) cycle. Additionally, PEG–PCL@Cur hindered the generation of inorganic phosphate (Pi), resulting in a decreased level of ATP production. These metabolic changes promoted apoptosis and enhanced melanoma cell death. In vivo, PEG–PCL@Cur significantly curtailed tumor growth in a melanoma xenograft model and displayed an enhanced biocompatibility. Histological analysis showed extensive tumor necrosis, diminished proliferation markers, and elevated levels of apoptosis indicators. Overall, our integrated metabolomic and proteomic approach highlights how PEG–PCL@Cur targets SDHA-mediated metabolic reprogramming and mitochondrial dysfunction. This discovery positions PEG–PCL@Cur as a promising nanoplatform for melanoma therapy and offers valuable insights into the development of targeted nanomedicines.

KEYWORDS: PEG–PCL, melanoma, curcumin, succinate dehydrogenase, metabolic reprogramming

1. INTRODUCTION

Melanoma, a highly aggressive skin cancer with rising global incidence, presents significant therapeutic challenges due to its resistance to conventional treatments and rapid progression.^{1–5} This resistance underscores the need for novel therapeutic approaches that can target the unique mechanisms driving melanoma growth and survival. In this context, natural compounds with anticancer properties have gained attention as potential alternatives to conventional therapies. Among these, curcumin, a naturally derived compound with well-documented anti-inflammatory and antitumor activities, has shown promise in oncology. Its ability to regulate tumor growth and progression through multiple molecular pathways makes it a valuable candidate for cancer treatment.^{6–10} Specifically, curcumin's diverse mechanisms of action offer potential to disrupt pathways critical for melanoma development. However, despite its therapeutic potential, the clinical application of curcumin is limited by poor solubility, chemical instability, and low bioavailability. These challenges reduce its effectiveness in vivo, highlighting the need for innovative strategies to enhance its delivery and efficacy in treating aggressive cancers like melanoma.^{11–15}

To address these limitations, nanotechnology-based drug delivery systems have emerged as a promising solution. These systems can improve the solubility, stability, and targeted delivery of therapeutic agents, thereby enhancing their clinical efficacy.^{16–20} Among them, polyethylene glycol–polycaprolactone (PEG–PCL) nanoparticles have attracted significant attention. This versatile platform offers improved drug bioavailability, reduced systemic toxicity, and enhanced therapeutic performance.^{21–25} Encapsulation of curcumin within PEG–PCL nanoparticles (PEG–PCL@Cur) has been proposed to overcome the major limitations of free curcumin such as poor solubility and low bioavailability. By improving these properties, PEG–PCL@Cur can enhance curcumin's antitumor potential. While PEG–PCL@Cur has shown promise in cancer therapy, reports on its application in melanoma treatment remain limited. Moreover, studies investigating the molecular mechanisms underlying its therapeutic effects against melanoma are particularly scarce.

Metabolic reprogramming, a defining feature of cancer cells, plays a crucial role in supporting melanoma growth and survival.^{26–30} As a metabolically dependent tumor, melanoma relies heavily on mitochondrial oxidative phosphorylation

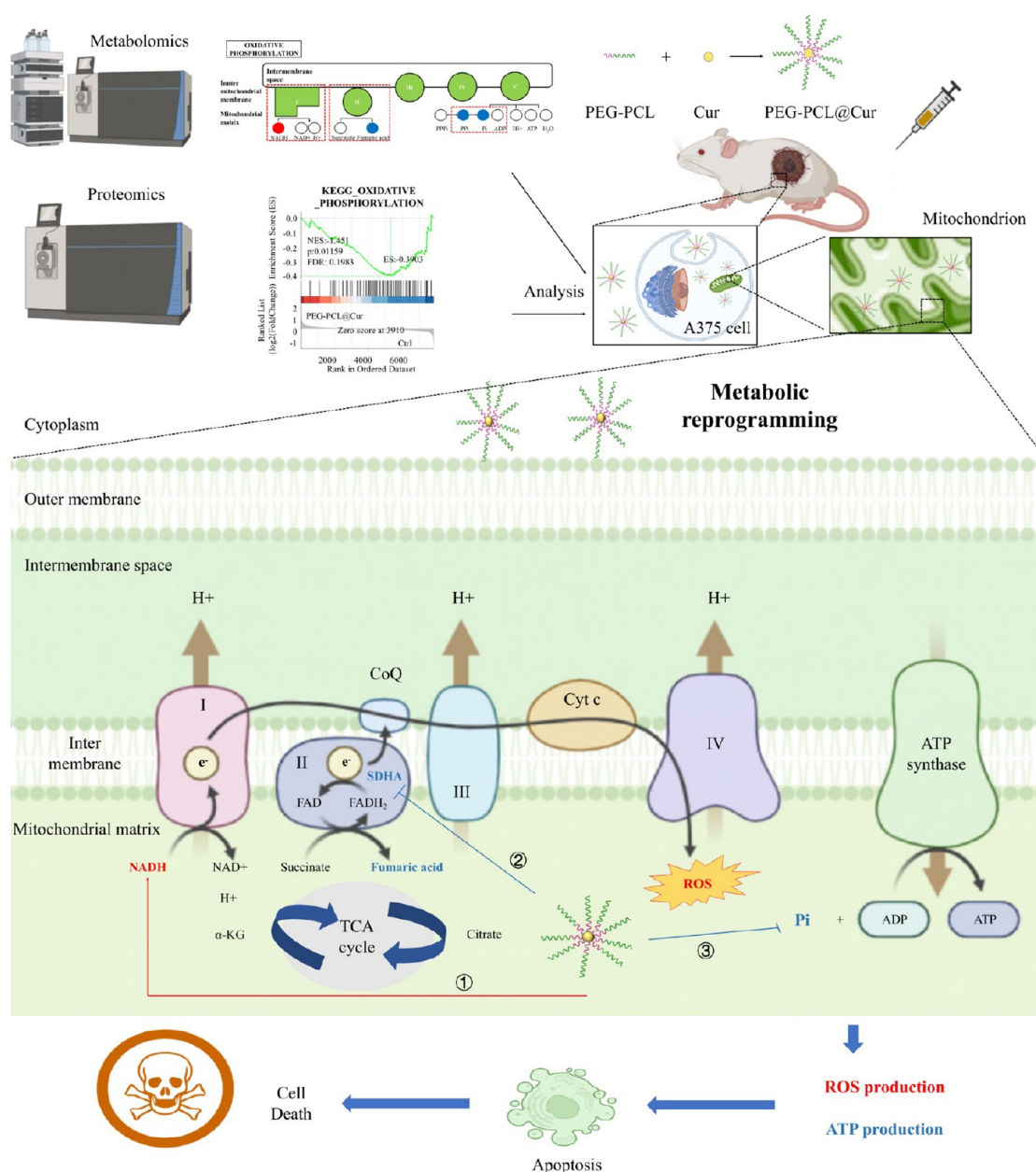
(OXPHOS) to meet its energy demands. Disruption of OXPHOS and related pathways, particularly those regulated by succinate dehydrogenase (SDHA), has been closely linked to melanoma progression.^{31–33} Given these characteristics, targeting metabolic reprogramming offers a promising approach for melanoma therapy. In this study, we utilized metabolomic and proteomic analyses to investigate how PEG–PCL@Cur nanoparticles interact with SDHA-mediated pathways. These nanoparticles were found to inhibit melanoma cell proliferation by disrupting the OXPHOS and inducing mitochondrial dysfunction, providing insights into their underlying mechanisms.

This work aims to bridge the gap between the mechanistic understanding and therapeutic application by investigating PEG–PCL@Cur in both in vitro and in vivo models of melanoma. Specifically, we evaluate the nanoparticles' ability to regulate metabolic reprogramming, disrupt mitochondrial integrity, and induce apoptosis through SDHA-mediated pathways. The findings of this study not only underscore the potential of PEG–PCL@Cur as an effective antimelanoma strategy but also provide a robust foundation for the development of targeted nanotherapeutics addressing metabolic vulnerabilities in cancer (Scheme 1).

2. RESULTS AND DISCUSSIONS

2.1. The Preparation and Characterization of APEG–PCL. Amphiphilic block copolymers are widely used as drug delivery vehicles in nanomedicine as they can enhance the solubility, reduce the side effects, and increase the accumulation of a wide range of hydrophobic drugs on the tumor side. It should be noted that biocompatibility and biodegradability are two important factors that must be considered when selecting a drug delivery vehicle. PEG and PCL have been approved by the FDA for use as biomaterials; therefore, PEG–PCL-based biomaterials have attracted a lot of attention. In this work, a PEG–PCL block copolymer was synthesized through a ring-opening polymerization of ϵ -CL by using APEG–OH as an initiator. The chemical structure of the obtained block copolymer was characterized by NMR and FTIR. Figure 1A shows the NMR analysis of PEG–PCL. The proton peaks attributed to the allyl group of the APEG block appeared in 5.8 and 5.3 ppm. The proton peak located in 3.5 to 3.7 belongs to the methylene group repeated unit of APEG. As for the PCL block, the chemical shift of the methylene groups was located at

Scheme 1. Schematic Illustration of the Mechanisms Underlying the Anticancer Effects of PEG–PCL-Loaded Curcumin Nanoparticles



4.0, 2.3, 1.6, and 1.4 ppm, respectively. By comparing the integrated area of the methyl group on the repeat unit of PCL with the allyl group of the APEG block, we were able to calculate the polymerization degree of PCL to be approximately 40 and the molecular weight to be approximately 4480 g/mol; therefore, the molar mass of the block copolymer was approximately 6880 g/mol. Furthermore, the chemical structure of APELG-PCL was also confirmed by the FTIR spectrum. As shown in Figure 1B, the characteristic absorption peaks of the PCL block appeared at 2930 and 2870 cm^{-1} ($\nu\text{C-H}$) and 1721 cm^{-1} ($\nu\text{C=O}$). The peaks at 1652 and 1547 cm^{-1} are ascribed to APEG block. The peak at 1461 cm^{-1} was attributed to the bending vibration of CH_2 , and the absorption peaks at the frequencies of 1100 cm^{-1} are attributed to the C–O stretching vibrational of the PEG segment, respectively.

2.2. Size and Morphology of Blank and Curcumin-Loaded Nanoparticles.

Amphiphilic block copolymers can self-assemble into nanoparticles, in which the hydrophobic blocks form the inner core and the hydrophilic block forms the hydrophilic layer. The self-assembly behavior of APEG–PCL in aqueous solution was characterized by DLS and TEM. As illustrated in Figure 1C,D, the diameter of the blank nanoparticles was approximately 120 nm, and spherical nanoparticles were confirmed by the TEM. It is interesting to note that the size of the curcumin-loaded nanoparticles reached to 125 nm (Figure 1C), which was larger than that of the blank nanoparticles. We attributed this phenomenon to the fact that hydrophobic curcumin was encapsulated in the hydrophobic core of the nanoparticles. From Figure 1D, we can find that the nanoparticles remain spherical even though the drug is encapsulated in the core. The drug loading capacity (DLC) of

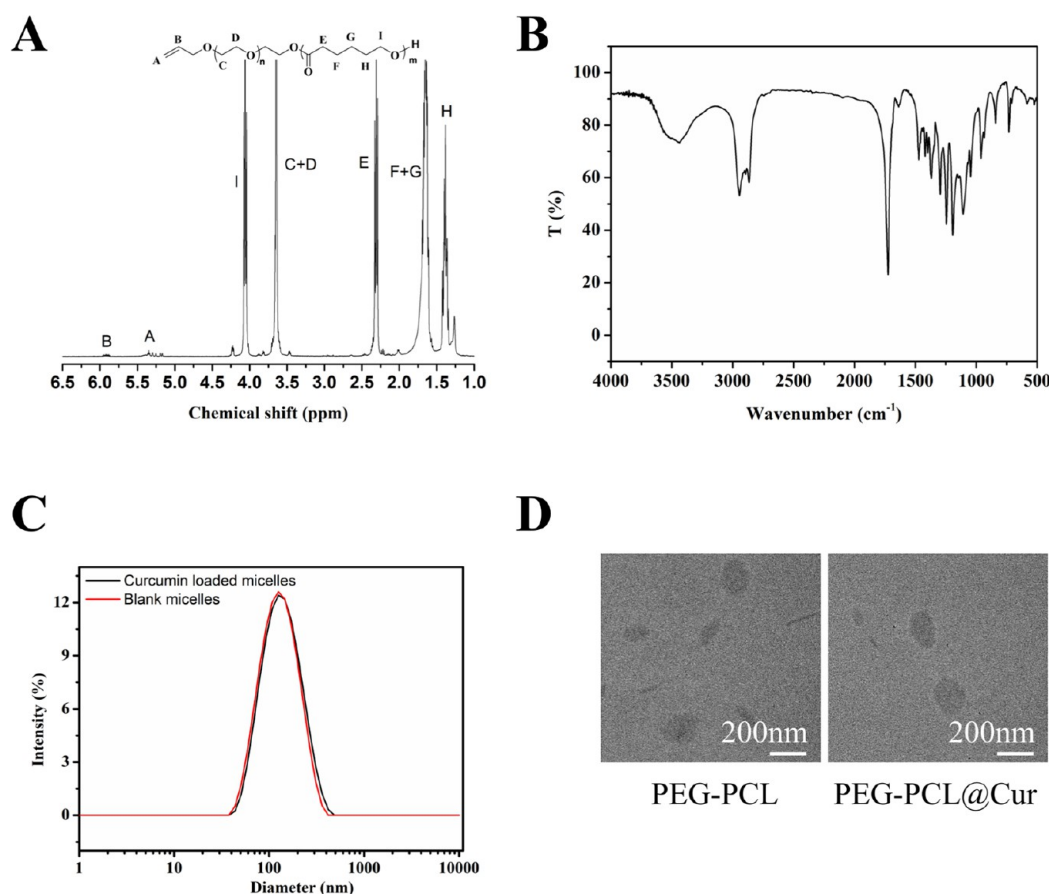


Figure 1. Physical characterization of PEG-PCL or PEG-PCL@Cur nanoparticles. (A) NMR spectrum of PEG-PCL. (B) FTIR spectrum of PEG-PCL. (C) Size distribution of PEG-PCL and PEG-PCL@Cur nanoparticles measured by DLS. (D) TEM images of PEG-PCL and PEG-PCL@Cur nanoparticles.

PEG-PCL@Cur was about 71.2%. The zeta potential of PEG-PCL was -6.27 mV, while that of PEG-PCL@Cur was -1.55 mV (Figure S2). Further, the release profile of curcumin from PEG-PCL@Cur micelles was evaluated by using an *in vitro* dialysis method. The release study was performed in PBS containing 10% serum to mimic physiological conditions. We also conducted a kinetic analysis using the first-order kinetics model. Our data indicate that the curcumin release follows the first-order kinetics model, with approximately 40.3% of the total curcumin released over 7 days (Figure S1).

2.3. Inhibitory Effects of PEG-PCL@Cur on A375 Cells *In Vitro*. The biocompatibility of the PEG-PCL nanodelivery system was evaluated by using the CCK-8 assay. No significant cytotoxicity was observed in A375 melanoma cells at various PEG-PCL concentrations (Figure 2A). This confirms the excellent biocompatibility of PEG-PCL, consistent with previous studies.^{34,35} To assess the antimelanoma effects of PEG-PCL@Cur, A375 cells were treated with PBS, Cur, or PEG-PCL@Cur at different concentrations for 24 and 48 h (Figure 2B). The CCK-8 assay showed that PEG-PCL@Cur was less toxic to A375 cells than free Cur. This aligns with earlier findings and highlights the advantages of nanomaterials in reducing toxicity while maintaining therapeutic effects. The difference arises from their distinct cellular entry mechanisms. Unlike free Cur, which passively diffuses into cells, nanomaterials like PEG-PCL@Cur use a more targeted internalization method. This makes them more effective at delivering drugs while reducing side effects. Mechanistically, the curcumin

delivery pathways differ significantly between free Cur and PEG-PCL@Cur nanoparticles.^{36–38} Free Cur enters cells via passive diffusion, whereas PEG-PCL@Cur relies on a targeted uptake mechanism. This targeted approach reduces systemic toxicity and enhances therapeutic efficacy. As a result, PEG-PCL@Cur achieves a balance between minimizing toxicity and maximizing treatment effects. Further functional assays demonstrated that PEG-PCL@Cur significantly inhibited A375 cell invasion and migration compared with controls, including free Cur (Figure 2C–F; Figures S3 and S4). This suggests that PEG-PCL@Cur is more effective at suppressing key processes involved in melanoma progression.

In summary, these findings provide strong evidence for the therapeutic potential of PEG-PCL@Cur nanoparticles as a promising antimelanoma strategy. By effectively targeting cell invasion and migration, PEG-PCL@Cur could offer a significant improvement in melanoma treatment.

2.4. Untargeted Metabolomics. Recent studies have shown that curcumin inhibits tumor cell growth by regulating metabolic reprogramming.^{39–42} Building on this premise, we demonstrated that polyethylene glycol–polycaprolactone nanoparticles loaded with curcumin (PEG-PCL@Cur) significantly suppressed the activity of A375 melanoma cells in cellular assays.

Untargeted metabolomics is widely used to examine how treatments affect cellular metabolism by identifying changes in metabolites across various pathways. This method provides valuable insights into metabolic shifts linked to disease or treatment responses.^{43–45} To investigate how PEG-PCL@Cur

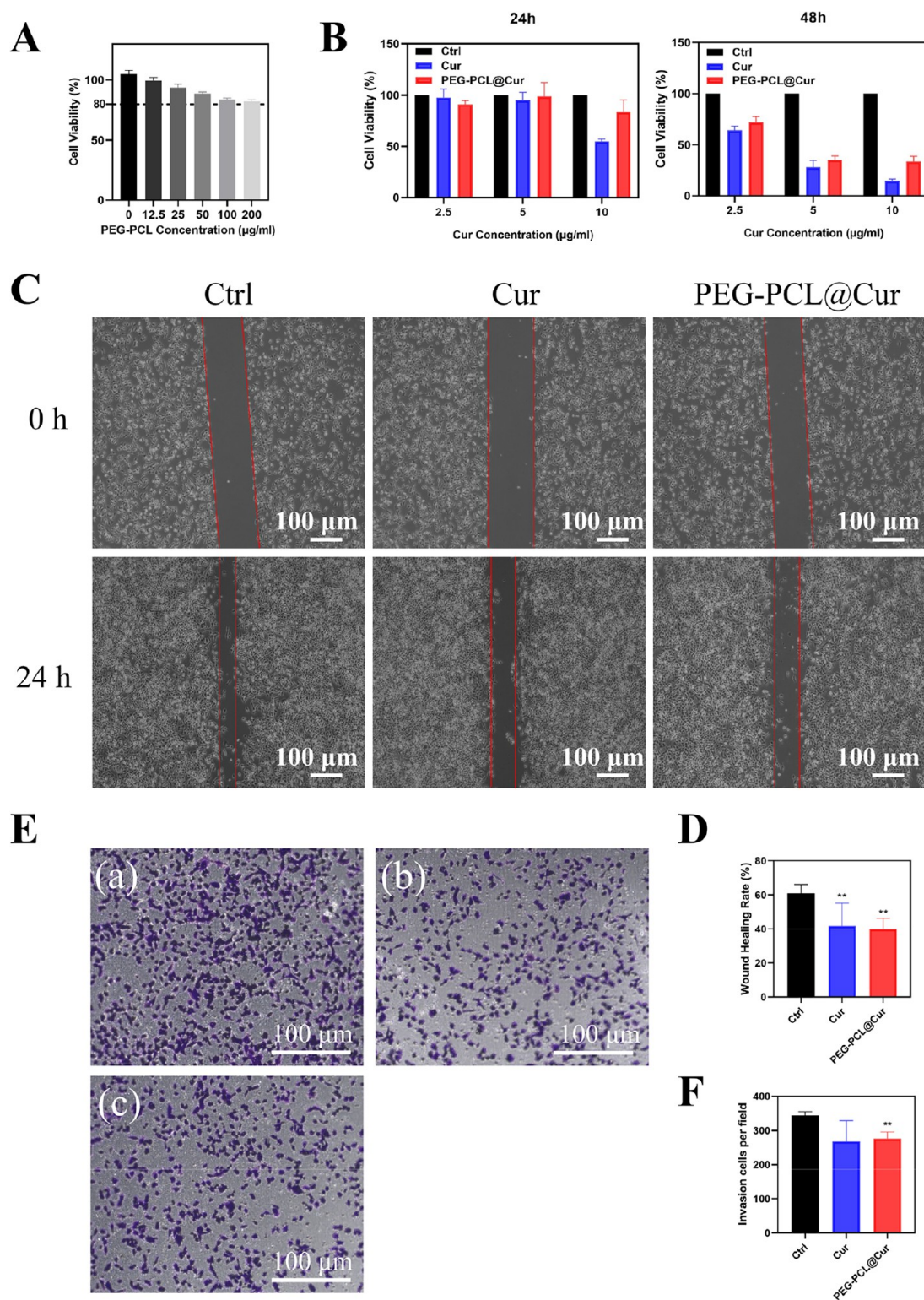


Figure 2. Biocompatibility and antimelanoma efficacy of nanoparticles on A375 cells. (A) Biocompatibility assessment after treatment with PEG-PCL. (B) Inhibitory effects on cell proliferation, (C, D) migration, and (E, F) invasion on A375 cells after treatment with PBS, Cur, or PEG-PCL@Cur. (a) represents the control group, (b) represents the Cur group, and (c) represents the PEG-PCL@Cur group. * $P < 0.05$, ** $P < 0.01$ compared to the control group; # $P < 0.05$, ## $P < 0.01$ compared to the Cur group.

impacts tumor metabolic reprogramming, we conducted untargeted metabolomics. The experimental setup included a control group (A) and a PEG-PCL@Cur-treated group (B), each with six replicates. Principal component analysis (PCA) was performed first, revealing clear separation between the two

groups under both positive and negative ion modes.⁴⁶ This indicated substantial changes in intracellular metabolites after PEG-PCL@Cur treatment. We then used orthogonal partial least-squares discriminant analysis (OPLS-DA) to simplify the model while improving interpretability and maintaining

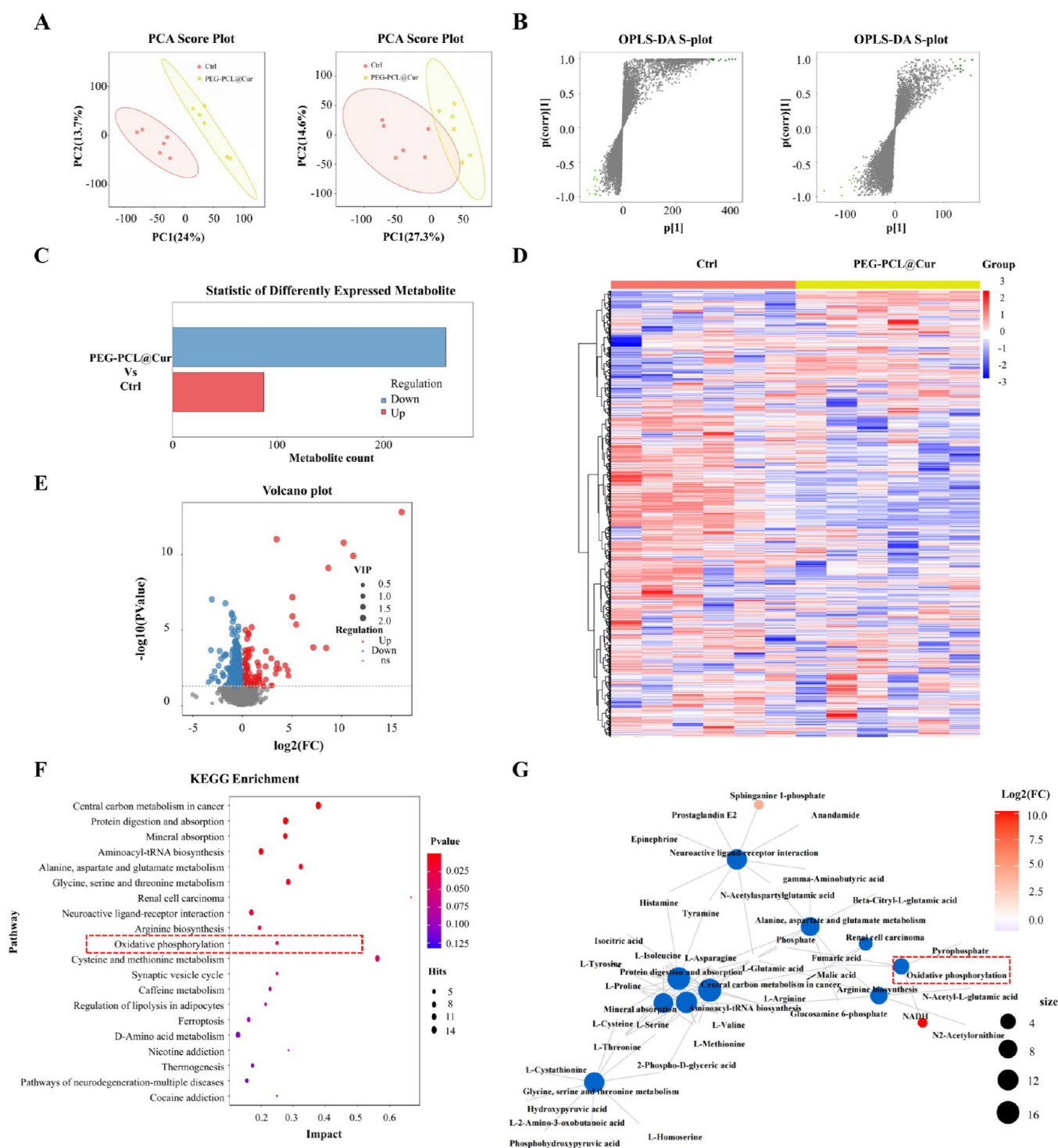


Figure 3. Effects of PEG-PCL@Cur nanoparticles on metabolic reprogramming of A375 cells. (A, B) PCA and OPLS-DA; (C) statistics of differently expressed metabolite; (D, E) heatmaps and volcano plots; (F) KEGG pathways; and (G) network diagram of the metabolite after treatment with PBS or PEG-PCL@Cur.

predictive accuracy.⁴⁷ S-plots derived from OPLS-DA identified metabolites with VIP values above 1 as the main contributors to group differences. As illustrated in Figure 3A,B, metabolites located closer to the primary axes were more significant. Based on quality control results, metabolites were identified using spectral databases like HMDB and KEGG.⁴⁸ Secondary structural confirmation was achieved by matching fragment ion data with the data in these databases. A total of 347 significantly altered metabolites were identified, including 87

that were upregulated and 260 that were downregulated (Figure 3C). Heatmaps and volcano plots were used to visualize these changes (Figure 3D,E). KEGG enrichment analysis revealed that the altered metabolites were mainly associated with pathways such as central carbon metabolism in cancer, protein digestion and absorption, mineral absorption, aminoacyl-tRNA biosynthesis, and alanine, aspartate, and glutamate metabolism (Figure 3F). Among these, mitochondrial oxidative phosphorylation is a well-studied pathway in cellular metabolic

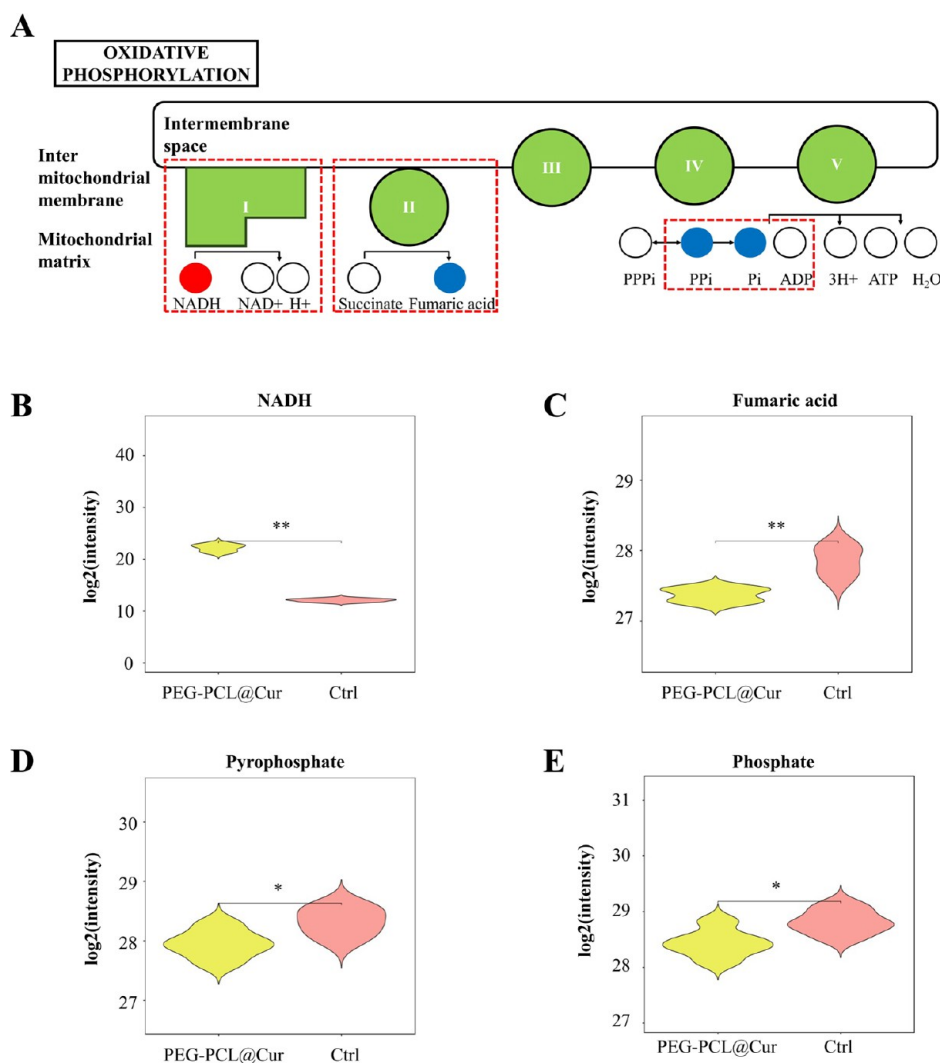


Figure 4. Metabolomics analysis of PEG-PCL@Cur in A375 Cells. (A) Metabolite changes in the oxidative phosphorylation pathway after PEG-PCL@Cur treatment. Metabolite levels of (B) NADH, (C) fumaric acid, (D) pyrophosphate, and (E) phosphate after treatment with PBS or PEG-PCL@Cur. * $P < 0.05$, ** $P < 0.01$ compared to control.

reprogramming.^{49,50} Among the 347 significantly altered metabolites, pathway enrichment analysis identified oxidative phosphorylation as one of the most prominently affected pathways, highlighting its critical role in tumor cell energy metabolism and survival. Given its well-established involvement in metabolic reprogramming and its potential as a therapeutic target, we prioritized this pathway for further investigation. As mitochondria are the main ATP producers, targeting oxidative phosphorylation to reduce ATP production is a potential strategy to inhibit tumor growth.^{51–55} Thus, oxidative phosphorylation was chosen for further investigation. In this pathway, fumaric acid, pyrophosphate, and phosphate levels were reduced, while NADH levels increased (Figure 3G). Violin plots were used to illustrate changes in key metabolites, and KEGG mapping highlighted fumaric acid as the most significantly decreased metabolite in the PEG-PCL@Cur-treated group (Figure 4A–E).

2.5. Proteomics. Recent advancements in proteomics have enabled the identification of differential proteins, offering essential insights into precision medicine and drug discovery.^{56–58} Building on our metabolomic findings, this study utilized proteomics to investigate the effects of PEG-PCL@Cur

on differential protein expression in A375 cells. This approach aimed to uncover potential molecular mechanisms. Proteomic analysis was conducted on two groups: the control (A) and PEG-PCL@Cur-treated (B) groups, with three replicates per group. PCA results showed clear separation between the groups (Figure S2), indicating significant protein expression changes after PEG-PCL@Cur treatment. Differential proteins were identified based on a fold change (FC) > 1.5 (upregulated) or < 0.67 (downregulated) and a P value < 0.05 . A total of 63 differential proteins were found, with 36 upregulated and 27 downregulated. These findings were visualized using volcano and heatmap plots (Figure 5A–C). To understand the biological roles of these proteins, Gene Ontology (GO) annotation categorized them into three domains: biological processes (BPs), molecular functions (MFs), and cellular components (CCs). Enriched BP terms included positive regulation of epithelial cell apoptosis, regulation of endothelial cell proliferation in sprouting angiogenesis, and inhibitory synapse assembly. Cellular components were linked to platelet alpha granule lumen and blood microparticles, while molecular functions included signaling receptor binding and neurotrophin binding (Figure S3). KEGG pathway (Figure S4) enrichment

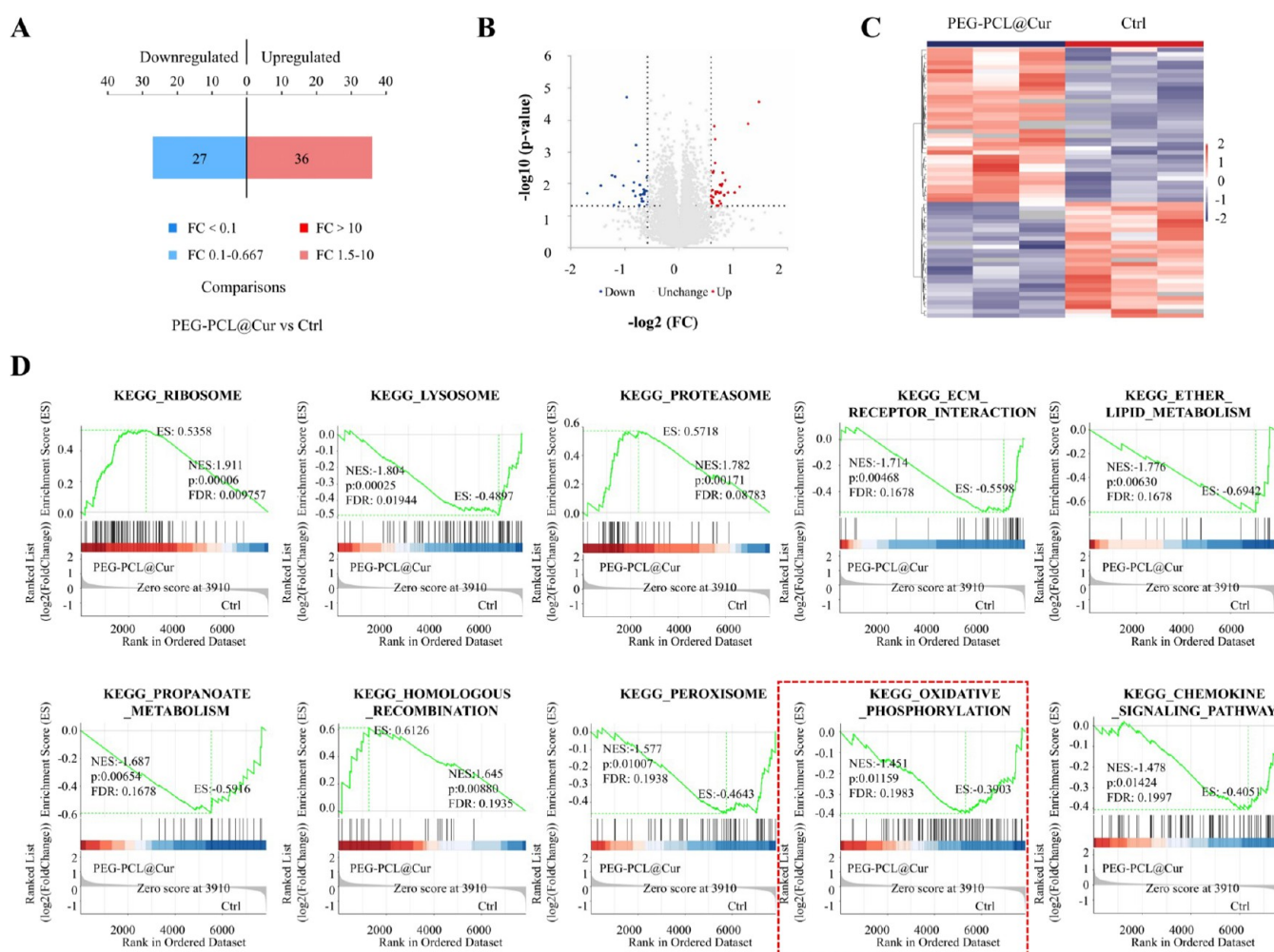


Figure 5. Proteomic analysis of the differential protein expression in A375 cells treated with PEG–PCL@Cur nanoparticles. (A) Histogram of downregulated and upregulated protein expression, (B) volcano plot, (C) heatmap, and (D) GSEA-KEGG analysis after treatment with PBS or PEG–PCL@Cur.

analysis highlighted significant impacts of PEG–PCL@Cur on pathways such as "Cytoskeleton in muscle cells", "Arrhythmogenic right ventricular cardiomyopathy", and "Viral myocarditis". However, KEGG analysis primarily emphasizes genes with pronounced changes, potentially overlooking subtle yet important alterations. To address this, Gene Set Enrichment Analysis (GSEA) was performed to assess overall gene expression trends.⁵⁹ GSEA identified 21 significantly altered pathways, with the top 10 presented in Figure 5D. Among these, the oxidative phosphorylation pathway was notably suppressed. By integrating metabolomic and proteomic analyses, we found that PEG–PCL@Cur significantly reduced fumaric acid levels, which is a crucial metabolite in A375 cells. Concurrently, key proteins involved in oxidative phosphorylation, including MT-CO3, SDHA, ATP5F1A, NDUFS4, and MT-ND4, were downregulated. Notably, SDHA, a critical enzyme in this pathway, showed a marked decrease in the level of expression. This reduction directly lowered fumaric acid levels and disrupted mitochondrial oxidative phosphorylation.^{60–63} These findings reveal that PEG–PCL@Cur mediates metabolic reprogramming in A375 cells by targeting SDHA-driven oxidative phosphorylation. This disruption suppresses tumor cell proliferation and the metabolic activity. The study provides mechanistic insights into how PEG–PCL@Cur regulates tumor

metabolism, offering a basis for developing innovative targeted therapies.

Our metabolomic analysis showed that PEG–PCL@Cur lowers fumaric acid and disrupts OXPHOS. In parallel, our proteomics data showed a clear decrease in SDHA, a key OXPHOS enzyme. This dual-omics approach proves that PEG–PCL@Cur targets SDHA-driven mitochondrial dysfunction. This, in turn, stops energy production and causes cell dysfunction in melanoma. These results show a strong link between the metabolomics and proteomics data. The fall in fumaric acid matches the drop in SDHA. This is supported by our KEGG analyses. In contrast to free curcumin, which mainly inhibits glycolysis,⁴⁰ our results show that PEG–PCL@Cur mainly stops OXPHOS by lowering SDHA. However, it differs from studies that focus on glycolysis inhibition in melanoma. Also, our data show that NADH builds up and leads to more ROS. This gives new insights into mitochondrial therapies. These combined findings support the use of PEG–PCL@Cur in clinical settings. They show that melanoma cells have a weakness in their metabolism.³⁰ PEG–PCL@Cur disrupts the emulsions of both OXPHOS and SDHA. This dual action is different from traditional treatments.

2.6. PEG–PCL@Cur Induces Mitochondrial Dysfunction and Apoptosis in A375 Cells via SDHA-Mediated

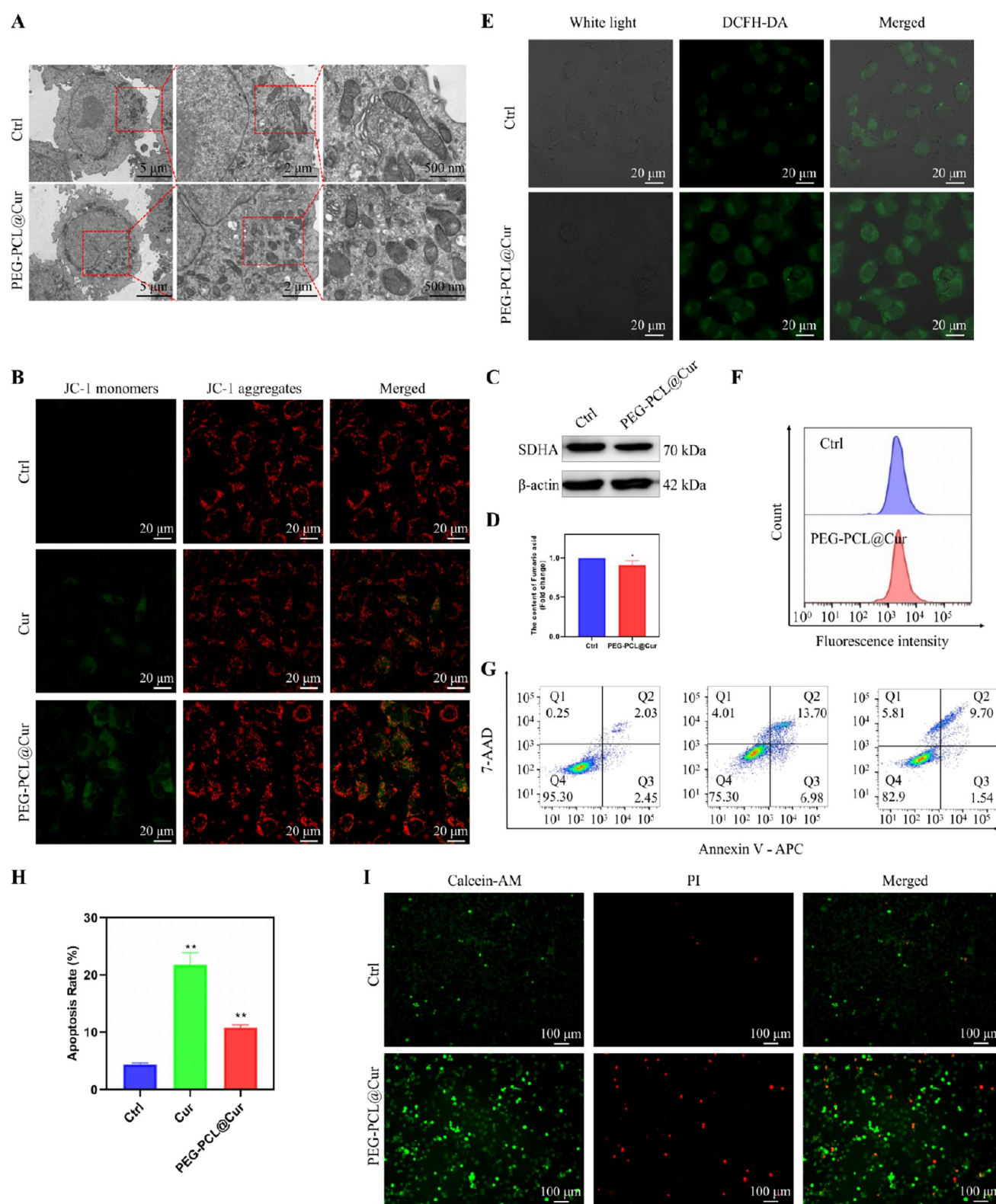


Figure 6. Effects of PEG-PCL@Cur nanoparticles on A375 cell mitochondria and metabolism. (A) TEM images of mitochondrial morphology, (B) $\Delta\psi_m$ measurements indicating mitochondrial function, (C, D) SDHA expression and fumaric acid levels, (E, F) ROS production analyzed by confocal microscopy and flow cytometry, (G, H) apoptosis rates determined, and (I) live/dead staining after treatment with PBS, Cur, or PEG-PCL@Cur. * $P < 0.05$, ** $P < 0.01$ compared to control group.

Metabolic Reprogramming. Building on the sequencing results, we examined whether PEG-PCL@Cur affects A375 cell growth by disrupting mitochondrial oxidative phosphorylation (OXPHOS). Transmission electron microscopy (TEM)

revealed significant mitochondrial changes in treated cells, such as swelling, rounding, and reduced cristae density, compared to the control group (Figure 6A). These abnormalities align with our metabolomic and proteomic findings. To

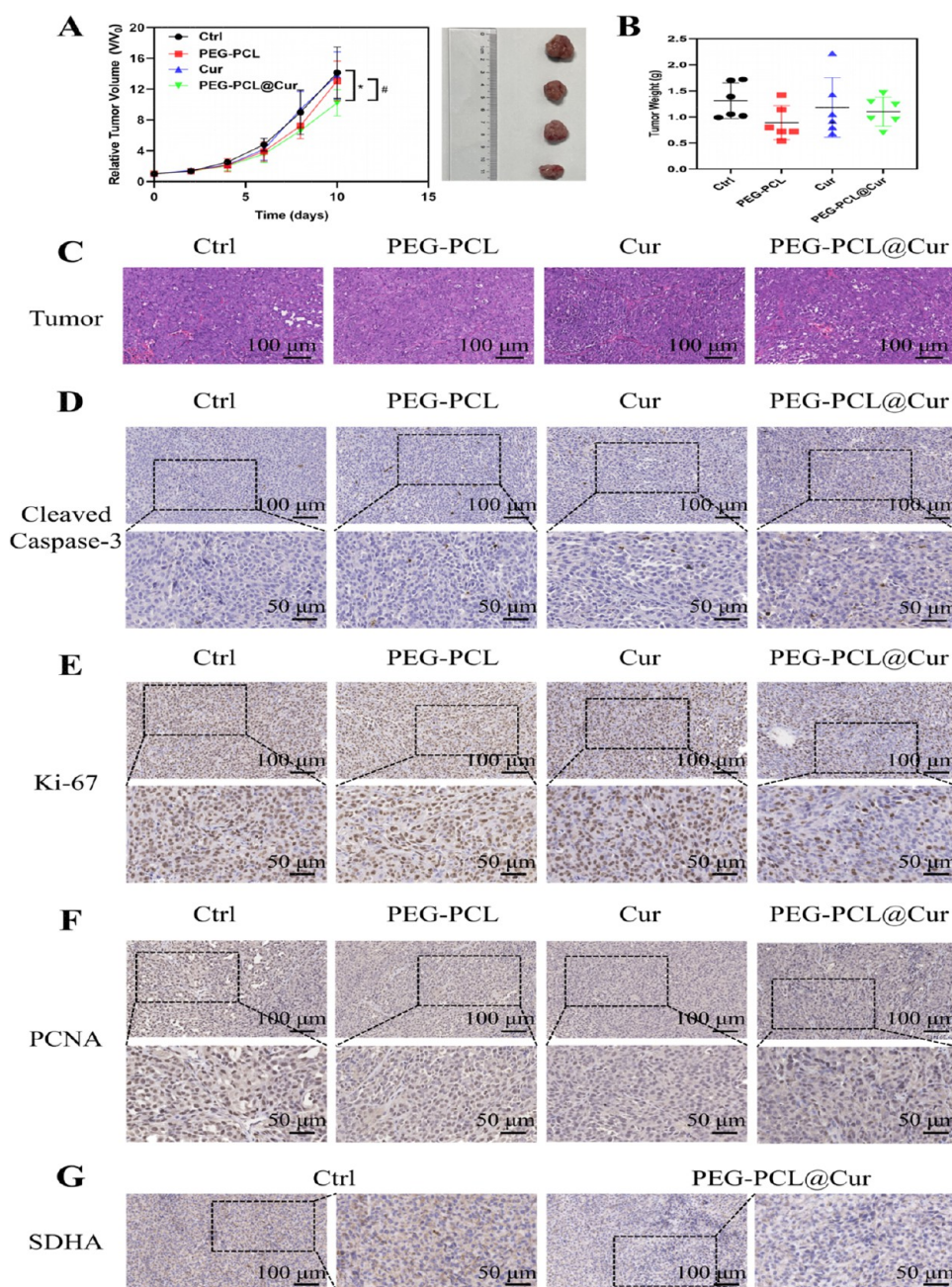


Figure 7. PEG-PCL@Cur nanoparticles suppress melanoma growth in vivo. (A) Relative tumor volume and (B) tumor weight in melanoma-bearing mice. (C) HE staining of tumor tissues. (D) Expression levels of cleaved caspase-3, (E) Ki-67, and (F) PCNA after treatment with PBS, PEG-PCL, Cur, or PEG-PCL@Cur and (G) SDHA in tumor tissues after treatment with PBS or PEG-PCL@Cur. * $P < 0.05$, ** $P < 0.01$ compared to the PBS group; # $P < 0.05$, ## $P < 0.01$ compared to the Cur group.

assess mitochondrial membrane potential ($\Delta\psi$ m), we used the JC-1 assay, a key measure of mitochondrial function.⁶⁴ Untreated cells displayed high $\Delta\psi$ m, forming red fluorescent J-aggregates. In contrast, treated cells showed increased green fluorescence due to monomeric JC-1 accumulation, indicating a marked reduction in the $\Delta\psi$ m (Figure 6B). In this experiment, free curcumin was included as a positive control group to provide a direct comparison with PEG-PCL@Cur.

To explore the role of SDHA and fumaric acid in this process, we measured SDHA expression through Western blotting and fumaric acid levels using the WST-8 assay. PEG-PCL@Cur significantly reduced both SDHA expression and fumaric acid levels (Figure 6C,D and Figure S8). Since SDHA catalyzes the

conversion of succinate to fumaric acid in the TCA cycle, a decrease in SDHA expression was accompanied by reduced fumaric acid levels, further confirming SDHA downregulation and its impact on mitochondrial metabolism. Our metabolomic data showed a corresponding depletion of fumaric acid, indicating that SDHA inhibition disrupts fumaric acid synthesis and further interrupts TCA cycle activity. This metabolic disruption is critical as melanoma cells rely heavily on mitochondrial oxidative phosphorylation for energy production. The reduced availability of fumarate weakens the TCA cycle, limiting ATP generation and making melanoma cells more vulnerable to metabolic stress. While our study provides evidence that PEG-PCL@Cur downregulates SDHA and

disrupts mitochondrial metabolism, we acknowledge that direct measurement of SDHA enzymatic activity and comprehensive quantification of TCA cycle intermediates would further strengthen the mechanistic link. These aspects will be explored in future studies.

To further assess mitochondrial function, we measured intracellular ATP levels, which rely on the TCA cycle activity. Our results showed a significant reduction in ATP production after PEG–PCL@Cur treatment, supporting the notion that SDHA downregulation disrupts mitochondrial energy metabolism (Figure S9). Additionally, our proteomics data confirmed a significant decrease in SDHA protein levels, reinforcing the link between PEG–PCL@Cur treatment and impaired mitochondrial function. This suggests that PEG–PCL@Cur induces metabolic reprogramming by targeting SDHA, leading to fumaric acid depletion and disrupting the TCA cycle, which ultimately compromises melanoma cell survival. These results suggest that PEG–PCL@Cur disrupts the metabolism of OXPHOS by targeting mitochondrial metabolism.

Additionally, PEG–PCL@Cur increased the NADH levels, leading to excessive ROS production. Simultaneously, reduced SDHA expression impaired fumaric acid synthesis, disrupting the TCA cycle. PEG–PCL@Cur also inhibited the production of inorganic phosphate, which is a critical substrate for ATP synthesis. This mitochondrial dysfunction led to reduced ATP levels, compromising cellular energy balance.^{65,66} Since melanoma cells are highly dependent on mitochondrial metabolism, this disruption further amplifies oxidative stress, exacerbating mitochondrial damage. Increased ROS production, a hallmark of mitochondrial damage, was confirmed using confocal microscopy and flow cytometry (Figure 6E,F and Figure S10). In these experiments, free curcumin was also included as a positive control group in Figure 6G,H to better assess the comparative effects on ROS-induced apoptosis. Furthermore, cleaved caspase-3 is a critical indicator of apoptosis, and our Western blot results demonstrate that PEG–PCL@Cur treatment significantly upregulates the expression of cleaved caspase-3 to induce apoptosis (Figure S11). The oxidative stress further impaired mitochondrial integrity and triggered apoptotic signaling pathways. Flow cytometry analysis confirmed that PEG–PCL@Cur induced apoptosis, ultimately causing cell death (Figure 6I). While our study provides evidence that PEG–PCL@Cur downregulates SDHA and disrupts mitochondrial metabolism, we acknowledge that direct measurement of SDHA enzymatic activity and comprehensive quantification of TCA cycle intermediates would further strengthen the mechanistic link. These aspects will be explored in future studies. In summary, PEG–PCL@Cur reprograms melanoma cell metabolism by inhibiting SDHA, leading to fumaric acid depletion and TCA cycle disruption. This metabolic vulnerability reduces ATP synthesis and increases oxidative stress, ultimately impairing the survival of melanoma cell survival. These results reveal a metabolic reprogramming mechanism underlying its therapeutic effects and provide insights into its biomedical applications.

While our findings provide strong evidence that PEG–PCL@Cur downregulates SDHA and disrupts mitochondrial metabolism, certain limitations should be acknowledged. We did not include normal or noncancerous cells as controls in our metabolomic analysis, which would help determine whether PEG–PCL@Cur selectively targets melanoma cells while preserving normal cell function. Future studies will incorporate normal human melanocytes for comparison. Further inves-

tigation of these pathways will provide a deeper understanding of the mechanisms underlying PEG–PCL@Cur-induced cell death. Addressing these aspects will enhance the translational potential of PEG–PCL@Cur in melanoma therapy.

2.7. In Vivo Antitumor Efficacy. To assess the antitumor effects of PEG–PCL@Cur in vivo, we used a subcutaneous melanoma xenograft mouse model. The mice were treated with PBS, PEG–PCL, Cur, or PEG–PCL@Cur at a Cur dose of 10 mg/kg. Tumor volume and body weight were recorded throughout the study. The PEG–PCL@Cur-treated group showed a significant reduction in tumor volume compared with the control and Cur-only groups (Figure 7A). However, no statistically significant reduction in tumor weight was observed (Figure 7B), which may be due to the limited sample size and statistical power. To enhance the statistical reliability, future studies will incorporate larger sample sizes. One limitation of this study is the use of the A375 melanoma cell line, which, although widely employed in melanoma research, does not fully capture human tumor heterogeneity. More clinically relevant models, such as patient-derived xenografts (PDX) or organoid models, would better reflect the complexity of human tumors. Future research will integrate these models to validate the efficacy of PEG–PCL@Cur in a more physiologically relevant setting.

This discrepancy between in vitro and in vivo results may be attributed to the differences in the tumor microenvironment and drug delivery. Based on the enhanced permeability and retention (EPR) effect, our nanoparticles can accumulate and remain in tumor tissues. However, the complex tumor microenvironment and heterogeneous nature of the EPR effect may result in suboptimal nanoparticle penetration and uneven drug distribution. Additionally, we did not measure the intratumoral drug concentration, which is a limitation of this study. Future work will address this by conducting pharmacokinetic (PK) and biodistribution studies to assess drug accumulation and retention within tumors. Moreover, due to the inherent immune deficiency in BALB/c nude mice, which lack functional T lymphocytes, this model is not suitable for investigating immune-related effects. As a result, the immunomodulatory effects of PEG–PCL@Cur nanoparticles were not evaluated in the current study. In future studies, we aim to use more appropriate models to explore these effects and better understand the potential of combining PEG–PCL@Cur with immunotherapies. Furthermore, to improve targeting efficiency, we plan to functionalize our nanoparticles with specific targeting ligands, such as PRAME peptides or antibodies, to enhance the active targeting of melanoma cells. Preliminary explorations will compare passive targeting via the EPR effect to these active targeting strategies.

Histological and immunohistochemical (IHC) analyses provided further insights into the treatment's effects. Hematoxylin–eosin (HE) staining revealed significant necrosis and nuclear damage in tumors from the PEG–PCL@Cur-treated group (Figure 7C). IHC analysis showed an increased expression of the apoptosis marker cleaved caspase-3 (Figure 7D and Figure S5). In contrast, the expression of proliferation markers Ki-67 and PCNA was reduced (Figures 7D,E and Figures S6 and S7).

Additionally, the expression of SDHA, which is associated with OXPHOS, was significantly reduced in tumors treated with PEG–PCL@Cur (Figure 7G and Figure S8). These findings align with the in vitro results. Together, they demonstrate that PEG–PCL@Cur inhibits melanoma growth by suppressing

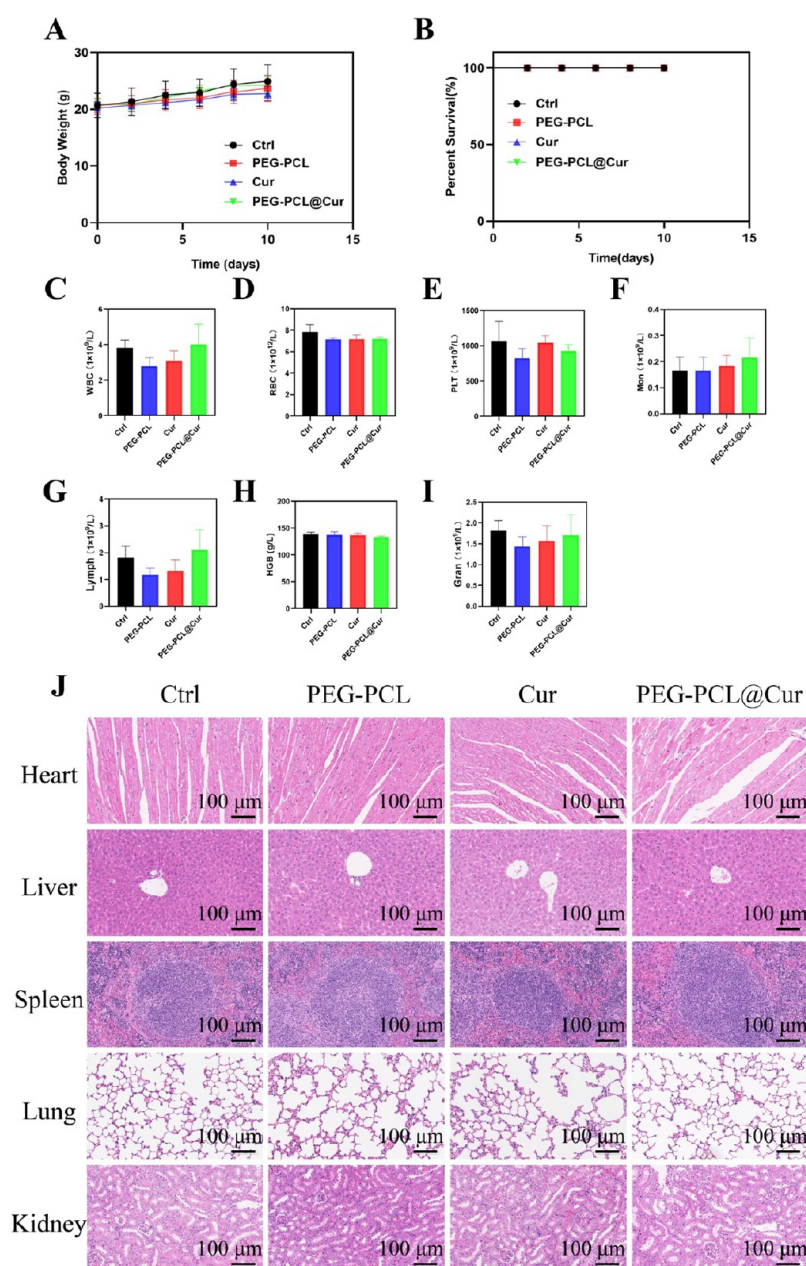


Figure 8. Evaluation of in vivo toxicity and biocompatibility. (A) Body weight and (B) survival rates of mice; (C–I) biochemical analysis of blood samples; and (J) hematoxylin and eosin staining of major organs, including the liver, spleen, lung, and kidney, after treatment with PBS, PEG–PCL, Cur, or PEG–PCL@Cur. * $P < 0.05$, ** $P < 0.01$ compared to the control group.

SDHA, disrupting mitochondrial function, and inducing apoptosis.

Although our study provides strong evidence of the antitumor effects of PEG–PCL@Cur, some limitations should be acknowledged. First, the lack of PK and biodistribution data makes it difficult to fully assess the targeting efficiency and retention of nanoparticles in tumor tissues. Future studies will address this by conducting detailed PK and biodistribution analyses. Second, the use of a single melanoma model (A375 xenografts) limits the generalizability of our findings. Future work will incorporate additional models, including PDX and melanoma organoids, to better mimic human tumor biology. Third, while our study highlights the role of SDHA suppression in PEG–PCL@Cur-mediated tumor inhibition, further mechanistic studies, such as metabolic flux analysis, will be needed to

elucidate the broader metabolic changes induced by this treatment. By addressing these limitations, future research will enhance the translational relevance of our findings and provide a more comprehensive understanding of PEG–PCL@Cur as a potential melanoma therapy.

2.8. In Vivo Biocompatibility Evaluation. The biocompatibility and safety of PEG–PCL@Cur were evaluated by examining the systemic toxicity and organ function in vivo. Mice injected intravenously with PEG–PCL@Cur showed no abnormalities in hematological parameters (Figure 8A–I). Histological analysis of major organs, including the liver, spleen, lungs, and kidneys, revealed no signs of toxicity or damage (Figure 8J). Additionally, the treated mice maintained a stable body weight and displayed a normal behavior throughout the

experiment. These findings confirm the excellent biocompatibility of PEG–PCL@Cur.

These results align with previous studies that also report the low toxicity and high safety of well-designed nanocarrier systems. For example, Behl et al.⁶⁷ demonstrated that PEG–PCL nanoparticles exhibit minimal toxicity in cancer models, while Grossen et al.⁶⁸ and Ferrentino et al.⁶⁹ reported excellent biocompatibility of PEG–PCL-based systems. Moreover, Jung et al.⁷⁰ provided evidence that such systems support safe cell transplantation. PEG–PCL@Cur demonstrated no systemic toxicity or adverse effects on organ health. Our study primarily focused on short-term toxicity, where we monitored biochemical parameters and histological changes shortly after treatment. However, we did not conduct long-term toxicity evaluations, especially regarding organs not assessed in our histological analysis, such as the brain, heart, and gastrointestinal system. We recognize that long-term effects on liver and kidney functions as well as potential alterations in immune responses remain unaddressed. We plan to conduct extended *in vivo* studies to evaluate the chronic toxicity of PEG–PCL@Cur nanoparticles. These studies include comprehensive analyses of liver enzymes, kidney function markers, and immune cell profiles over a longer period. This will help to establish the long-term safety and suitability of PEG–PCL@Cur for prolonged therapeutic use. This strong safety profile highlights its potential for clinical use. It also underscores the importance of designing nanocarriers that minimize side effects while ensuring therapeutic benefits.

3. CONCLUSIONS

In conclusion, this study reveals the diverse mechanisms by which PEG–PCL@Cur achieves its therapeutic effects in melanoma by targeting metabolic reprogramming. PEG–PCL@Cur exhibited strong *in vitro* antimelanoma activity by inhibiting the invasion and migration of A375 cells. Mechanistically, PEG–PCL@Cur increased ROS production by upregulating NADH levels and suppressing SDHA expression. This suppression reduced the fumaric acid levels and disrupted the TCA cycle. Additionally, PEG–PCL@Cur inhibited Pi generation, which decreased intracellular ATP levels. These combined effects triggered apoptosis and ultimately caused cell death. Untargeted metabolomics and proteomics further validated these findings. They confirmed the inhibition of mitochondrial oxidative phosphorylation. *In vivo* experiments supported these results, showing significant tumor volume reduction and histological evidence of apoptosis and metabolic disruption. Overall, PEG–PCL@Cur demonstrates great potential as a nanotherapeutic platform that modulates mitochondrial function and metabolic pathways. Future work should focus on improving tumor-targeting strategies to increase their effectiveness and explore their potential for clinical translation.

4. EXPERIMENTAL SECTION

4.1. Materials and Reagents. Stannous octoate and allyloxypolyethyleneglycol (APEG–OH, average M_n 2400) were purchased from Aladdin. Calcium hydride (CaH_2) was obtained from Alfa Aesar and used as received without further purification. ϵ -Caprolactone (ϵ -CL, 98%) was from Sigma-Aldrich and purified by distillation with calcium anhydride under reduced pressure. All other chemical reagents were used as received.

The schematic diagram was created using Biorender. Curcumin was purchased from KKL (USA). The A375 cell line was procured from the American Type Culture Collection (ATCC, USA). Cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) (Gibco,

USA) and 1% streptomycin/penicillin (Meilunbio, Dalian, China). The Cell Counting Kit-8 (CCK-8) was sourced from Meilunbio (Dalian, China). For Western blot analysis, rabbit anti-SDHA (1:1000, CST, USA) and mouse anti- β -actin (1:20,000, Proteintech Group, China) were used. Immunohistochemistry (IHC) employed the following antibodies: rabbit anti-Ki67 (1:1000, Servicebio, China), rabbit anti-PCNA (1:500, Servicebio, China), rabbit anti-cleaved caspase-3 (1:500, Servicebio, China), and rabbit anti-SDHA (1:2000, Servicebio, China). All other chemical reagents used in this study were of analytical reagent grade and utilized in their as-received condition.

4.2. The Preparation and Characterization of Allyloxypolyethyleneglycol–Poly(ϵ -caprolactone) Block Copolymer. The APEG–PCL block copolymer was synthesized via a one-step ring-opening polymerization of ϵ -CL in the presence of the initiator APEG–OH and the catalyzer stannous octoate. In a typical reaction, APEG–OH (2.4 g, 1 mmol) was first added into the Schlenk reactor equipped with a stirring bar under an argon atmosphere. To remove the water, the Schlenk reactor was sealed with a rubber plug, placed in an oil bath, and depressurized at 80 °C for 1 h. ϵ -CL (4.8 g, 42 mmol) was added to the Schlenk reactor via a syringe under the protection of argon. Finally, after the addition of the catalyzer stannous octoate into the mixture, the temperature of the oil bath was set to 105 °C to start the ROP of ϵ -CL. After 8 h, the reaction was quenched by immersing the Schlenk reactor into ice–water. The mixture was dissolved in tetrahydrofuran (THF) and precipitated in hexane. The white precipitate was collected and dried in a vacuum drying oven overnight. The Fourier transform infrared spectroscopy of the block copolymer was recorded on a PerkinElmer Paragon 1000 infrared spectrometer. The chemical structure of mPEG–PCL was analyzed using a Bruker NMR instrument (Bruker AV-500), and deuteriochloroform was used as a solvent.

4.3. Preparation and Characterization of Blank Nanoparticles. For the preparation of the blank nanoparticles, 10 mg of APEG–PCL was dissolved in 1 mL of DMSO, and then the mixture was added into 5 mL of deionized water under continuous stirring. Subsequently, the mixture was transferred to a dialysis tube and dialyzed against deionized water for 24 h. The size of the obtained nanoparticles was recorded on a Malvern particle size analyzer. The dried state morphology of the nanoparticles was examined with a JEOL JEM-2100F transmission electron microscope.

4.4. Curcumin Loading and *In Vitro* Release. Ten milligrams of APEG–PCL and 1 mg of curcumin were dissolved in warm DMSO, and the resulting mixture was dropped into deionized water with continuous stirring. Following this, the solution was decanted into a dialysis tube and subjected to dialysis against deionized water for a period of 24 h. The resulting dialysate was then passed through a needle filter. The size and the dried state morphology of the curcumin-loaded nanoparticles were recorded on a Malvern particle size analyzer and a JEOL JEM-2100F transmission electron microscope, respectively. The concentration of curcumin was determined using an established standard curve, and the drug loading content (DLC) was calculated using the following formula:

$$\text{DLC (\%)} = w_1/w_2 \times 100 \quad (1)$$

where w_1 is the weight of curcumin loaded in the nanoparticles and w_2 represents the weight of curcumin.

In vitro drug release was conducted as follows: 3 mL of the drug-loaded nanoparticle solution was placed into a dialysis bag and transferred to a centrifuge tube containing 27 mL of PBS. The tubes were then sealed and placed into a 37 °C water bath, where they were agitated at 100 rpm. At predetermined time intervals, 3 mL of solution outside the dialysis bag was withdrawn and replaced with 3 mL of fresh buffer solution. The drug release experiment was repeated three times, and the curcumin concentration was measured at 420 nm using a UV–vis spectrometer (UV-3150, Shimadzu, Japan). The accumulated percentage of released curcumin was calculated using the following equation:

$$E_r (\%) = \frac{V_e \sum_{i=1}^{n-1} C_i + V_0 C_n}{m_{\text{curcumin}}} \times 100\% \quad (2)$$

in which m_{curcumin} denotes the mass of curcumin within the micelles, V_0 signifies the total volume of the release medium (V_0 is 30 mL), V_e is the volume of the medium exchanged ($V_e = 3$ mL), and C_i is the concentration of curcumin in the i th aliquot.

4.5. CCK-8 Assay. A375 cells were exposed to PBS, Cur, or PEG–PCL@Cur at curcumin concentrations of 0, 2.5, 5, and 10 $\mu\text{g/mL}$. Following 24 h of incubation, 10 μL of the CCK-8 reagent was added to each well, and the plates were incubated for an additional hour. Afterward, absorbance was measured at 450 nm to determine cell viability, which was calculated using the following formula:

$$\text{cell viability (\%)} = (\text{OD}_{\text{exp}} - \text{OD}_{\text{blank}}) / (\text{OD}_{\text{ctrl}} - \text{OD}_{\text{blank}})$$

4.6. Transwell Assay. To assess the invasive behavior, A375 cells treated with PBS, Cur, or PEG–PCL@Cur were seeded in the Matrigel-coated upper chambers of a Transwell system. After 24 and 48 h of culture, cells that invaded through the membrane were fixed, stained, and imaged using a slide scanner for further analysis.

4.7. Wound Healing Assay. Cells were seeded at 5×10^5 cells per well in six-well plates and grown to approximately 90% confluence. A scratch wound was introduced using a pipet tip under sterile conditions followed by treatment with a serum-free medium containing PBS, Cur, or PEG–PCL@Cur. After 24 and 48 h, wound closure was monitored by capturing images for quantitative analysis.

4.8. Untargeted Metabolomics Analysis. Untargeted metabolomics was performed to assess PEG–PCL@Cur-induced metabolic reprogramming in A375 cells. Raw spectral data were converted to mzXML format using MSConvert, and peak detection, alignment, and quantification were conducted with XCMS using optimized parameters. Total ion normalization corrected system variability. Metabolites were identified based on accurate m/z ratios and molecular formulas using HMDB and KEGG, with fragment ion matching for confirmation. Metabolites within 30 ppm deviation were selected for analysis. Statistical analysis via principal component analysis (PCA) and orthogonal partial least-squares discriminant analysis (OPLS-DA) distinguished control and treatment groups, with key metabolites identified by variable importance in projection (VIP) scores > 1 and P values < 0.05 . KEGG pathway analysis linked differential metabolites to biological processes, visualized using KEGG Mapper and heatmaps.

4.9. Proteomics Analysis. Proteomics was used to analyze protein expression changes in A375 cells treated with PEG–PCL@Cur. Protein identification was based on accurate m/z ratios and peptide fragmentation patterns, using UniProt and NCBI databases, with proteins identified at $< 1\%$ FDR. Differentially expressed proteins were selected based on a > 1.5 -fold increase or < 0.67 -fold decrease and P value < 0.05 . PCA was performed to visualize group differences. Key proteins were identified using VIP scores > 1 . Functional enrichment was performed with GO, KEGG, and GSEA KEGG pathway analysis.

4.10. Live/Dead Staining. To assess cell viability, A375 cells were seeded in six-well plates and treated with PBS, Cur, or PEG–PCL@Cur. Subsequently, Calcein-AM and PI staining solutions were added followed by incubation at room temperature in the dark for 45 min. Afterward, the staining solution was removed to terminate the reaction. Cells were visualized under a fluorescence microscope for live and dead cell analysis.

4.11. ROS Detection. A375 cells were seeded at 5×10^5 cells per well in six-well plates and treated with PBS, Cur, or PEG–PCL@Cur. To detect ROS levels, cells were incubated with DCFH-DA at 37°C for 30 min. After incubation, the cells were washed three times with PBS and harvested by trypsinization. ROS generation was analyzed by using a confocal laser scanning microscope or flow cytometry.

4.12. Apoptosis Analysis. To assess apoptosis, A375 cells were seeded at 5×10^5 cells per well in six-well plates and treated with PBS, Cur, or PEG–PCL@Cur. The cells were stained with Annexin V-APC and 7-AAD by using a commercial apoptosis detection kit. Following staining, samples were analyzed by flow cytometry, and the data were further processed using the FlowJo software for quantitative evaluation.

4.13. JC-1. A375 cells were seeded in six-well plates and treated with PBS, Cur, or PEG–PCL@Cur. Cells were incubated with 1 mL of JC-1

staining solution at 37°C for 20 min, washed twice with $1\times$ JC-1 buffer, and resuspended in 2 mL of the culture medium. The mitochondrial membrane potential was analyzed via confocal laser scanning microscopy.

4.14. Western Blot Analysis. Western blotting was performed to quantify the SDHA and β -actin expression in A375 cells treated with PBS, Cur, or PEG–PCL@Cur. Proteins were extracted and quantified by using a BCA kit, separated by SDS-PAGE, and transferred onto PVDF membranes. Subsequently, the membranes were probed with specific primary antibodies followed by secondary antibody incubation. Signal visualization was achieved by using a chemiluminescent substrate.

4.15. Animal Experiments. The animal use protocol was reviewed and approved by the Animal Ethics and Welfare Committee of the Guangzhou Forevergen Biosciences Laboratory. In vivo experiments were conducted using BALB/c nude mice (4–6 weeks old) in compliance with ethical guidelines (Approval IACUC-AEWC-F240910021). A375 cells were suspended in a matrix gel and injected subcutaneously into the mice. When tumor volumes reached $50\text{--}100\text{ mm}^3$, the mice were randomly divided into four groups: control, PEG–PCL, Cur, and PEG–PCL@Cur. To ensure a fair comparison between free Cur and PEG–PCL@Cur, the curcumin dose was kept constant at 10 mg/kg for both Cur and PEG–PCL@Cur groups. Treatments were administered intravenously via tail vein injections, and tumor volume and body weight were measured at regular intervals. At the study end point, the animals were euthanized, and tumors and organs were harvested for analysis.

4.16. Immunohistochemistry. IHC analysis was performed to detect Ki-67, PCNA, cleaved caspase-3, and SDHA expression in tumor tissues. After deparaffinization and antigen retrieval, the sections were incubated with primary and secondary antibodies. Protein expression was visualized with DAB staining and counterstained with hematoxylin for microscopic evaluation.

4.17. Hematoxylin–Eosin Staining. Tumor tissues and major organs (heart, liver, spleen, lung, and kidney) were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned. These sections were stained with hematoxylin and eosin, dehydrated, and mounted using neutral balsam. Morphological changes were evaluated by microscopy or slide scanning.

4.18. Statistical Analysis. Statistical comparisons were performed using either the Student's t test or one-way ANOVA, with quantitative data represented as mean values $\pm$ standard deviation (SD).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsami.5c02854>.

Additional experimental data, including characterization of PEG–PCL@Cur nanoparticles, in vitro release profile, zeta potential, cell assays, proteomic analysis, and relevant protein expression levels; in vitro release profile of curcumin from PEG–PCL@Cur (Figure S1); zeta potential of PEG–PCL and PEG–PCL@Cur nanoparticles (Figure S2); wound healing assay and rate analysis of A375 cells treated with PBS, Cur, or PEG–PCL@Cur (Figure S3); invasion assay and analysis of A375 cells treated with PBS, Cur, or PEG–PCL@Cur (Figure S4); PCA of proteomic analysis in A375 cells treated with PBS or PEG–PCL@Cur (Figure S5); GO analysis of proteomic data in A375 cells: (A) BP, (B) MF, (C) CC (Figure S6); KEGG pathway analysis of proteomic data in A375 cells (Figure S7); ROS levels in A375 cells treated with PBS or PEG–PCL@Cur (Figure S8); SDHA protein expression in A375 cells treated with PBS or PEG–PCL@Cur (Figure S9); ATP content in A375 cells treated with PBS or PEG–PCL@Cur (Figure S10); (A) Western blot assay and (B) cleaved caspase-3

expression in A375 cells treated with PBS, Cur, or PEG–PCL@Cur (Figure S11); cleaved caspase-3 expression after treatment (Figure S12); Ki-67 expression after treatment (Figure S13); PCNA expression after treatment (Figure S14); and SDHA expression after treatment with PBS or PEG–PCL@Cur (Figure S15) (PDF)

AUTHOR INFORMATION

Corresponding Authors

Weide Zhong – Department of Urology, the Second Affiliated Hospital, School of Medicine, South China University of Technology, Guangzhou, Guangdong 510180, China; Guangdong Provincial Key Laboratory of Urology, the First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University, Guangzhou, Guangdong 510230, China; Guangzhou Laboratory, Guangzhou, Guangdong 510005, China; State Key Laboratory of Quality Research in Chinese Medicine, Macau University of Science and Technology, Taipa, Macau 999078, China; Guangdong Provincial Institute of Nephrology, Nanfang Hospital, Southern Medical University, Guangzhou, Guangdong 510515, China; Phone: +86 020 81048265; Email: zhongwd2009@live.cn

Huikang Yang – Department of Burn and Plastic Surgery, the Second Affiliated Hospital, School of Medicine and Department of Radiology, the Second Affiliated Hospital, School of Medicine, South China University of Technology, Guangzhou, Guangdong 510180, China; Phone: +86 020 81048265; Email: eyyanghk@scut.edu.cn

Ronghua Yang – Department of Burn and Plastic Surgery, the Second Affiliated Hospital, School of Medicine, South China University of Technology, Guangzhou, Guangdong 510180, China; orcid.org/0000-0002-9685-7003; Phone: +86 020 81048265; Email: 21720091@qq.com

Authors

Yixun Zhang – Department of Burn and Plastic Surgery, the Second Affiliated Hospital, School of Medicine, South China University of Technology, Guangzhou, Guangdong 510180, China

Xin Zhou – Department of Burn and Plastic Surgery, the Second Affiliated Hospital, School of Medicine, South China University of Technology, Guangzhou, Guangdong 510180, China

Wenlian Zheng – Department of Burn and Wound Repair, Shaoguan First People's Hospital, Shaoguan, Guangdong 512023, China

Jianwei Chen – Department of Burn and Plastic Surgery, the Second Affiliated Hospital, School of Medicine, South China University of Technology, Guangzhou, Guangdong 510180, China

Qianfeng Xu – Department of Urology, the Second Affiliated Hospital, School of Medicine, South China University of Technology, Guangzhou, Guangdong 510180, China

Zhenguo Liang – Guangdong Provincial Key Laboratory of Urology, the First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University, Guangzhou, Guangdong 510230, China

Hongli Zhang – Department of Burn and Plastic Surgery, the Second Affiliated Hospital, School of Medicine, South China University of Technology, Guangzhou, Guangdong 510180, China

Jundong Lin – Guangdong Provincial Key Laboratory of Urology, the First Affiliated Hospital of Guangzhou Medical

University, Guangzhou Medical University, Guangzhou, Guangdong 510230, China; Guangzhou Laboratory, Guangzhou, Guangdong 510005, China; orcid.org/0000-0001-7068-2172

Wenjie Xie – Department of Urology, the Second Affiliated Hospital, School of Medicine, South China University of Technology, Guangzhou, Guangdong 510180, China

Yangjia Zhuo – Department of Urology, the Second Affiliated Hospital, School of Medicine, South China University of Technology, Guangzhou, Guangdong 510180, China; orcid.org/0000-0003-3061-7303

Muqi Chen – Department of Urology, the Second Affiliated Hospital, School of Medicine, South China University of Technology, Guangzhou, Guangdong 510180, China

Fen Zou – Department of Urology, the Second Affiliated Hospital, School of Medicine, South China University of Technology, Guangzhou, Guangdong 510180, China

Biyan Wen – Guangdong Provincial Key Laboratory of Urology, the First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University, Guangzhou, Guangdong 510230, China

Zeheng Tan – Guangdong Provincial Institute of Nephrology, Nanfang Hospital, Southern Medical University, Guangzhou, Guangdong 510515, China

Complete contact information is available at:

<https://pubs.acs.org/10.1021/acsami.5c02854>

Author Contributions

[†]Y.Zhang, X.Z., W.Zheng, and J.C. contributed equally to this work.

Author Contributions

R.H.Y., H.K.Y., and W.D.Z. conceived the work. This manuscript was written by Y.X.Z., X.Z., W.L.Z., and J.W.C. Experiments were carried out by Q.F.X., Z.G.L., H.L.Z., J.D.L., W.J.X., and Y.J.Z. M.Q.C., F.Z., B.Y.W., and Z.H.T. analyzed the data. All authors have approved the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We acknowledge the technical support provided by Biorender for the creation of the schematic diagrams presented in this manuscript. This study was funded by the Chongqing Traditional Chinese Medicine Inheritance and Innovation team project (2023090006KJZX2022WJW008), the Guangdong Basic and Applied Basic Research Foundation (2022A1515012160, 2023A1515012320, and 2024A1515010477), the National Natural Science Foundation of China (82072813, 82272276, and 82373166), and the Science and Technology Development Fund, Macau SAR (0031/2021/A and 0090/2022/A).

REFERENCES

- (1) DeSantis, C. E.; Lin, C. C.; Mariotto, A. B.; Siegel, R. L.; Stein, K. D.; Kramer, J. L.; Alteri, R.; Robbins, A. S.; Jemal, A. Cancer treatment and survivorship statistics, 2014. *CA Cancer J. Clin* **2014**, *64* (4), 252–271.
- (2) Lopes, J.; Rodrigues, C. M.; Gaspar, M. M.; Reis, C. P. Melanoma management: from epidemiology to treatment and latest advances. *Cancers* **2022**, *14* (19), 4652.

- (3) Ahmed, B.; Qadir, M. I.; Ghafoor, S. Malignant Melanoma: Skin Cancer-Diagnosis, Prevention, and Treatment. *Critical reviews in eukaryotic gene expression* **2020**, *30* (4), 291–297.
- (4) Kim, H. J.; Kim, Y. H. Molecular Frontiers in Melanoma: Pathogenesis, Diagnosis, and Therapeutic Advances. *International Journal of Molecular Sciences* **2024**, *25* (5), 2984.
- (5) Khan, N. H.; Mir, M.; Qian, L.; Baloch, M.; Ali Khan, M. F.; Rehman, A. u.; Ngowi, E. E.; Wu, D. D.; Ji, X. Y. Skin cancer biology and barriers to treatment: Recent applications of polymeric micro/nanostructures. *J. Ad. Res.* **2022**, *36*, 223–247.
- (6) Joshi, P.; Joshi, S.; Semwal, D.; Bisht, A.; Paliwal, S.; Dwivedi, J.; Sharma, S. Curcumin: an insight into molecular pathways involved in anticancer activity. *Mini Rev. Med. Chem.* **2021**, *21* (17), 2420–2457.
- (7) Kunnumakkara, A. B.; Bordoloi, D.; Harsha, C.; Banik, K.; Gupta, S. C.; Aggarwal, B. B. Curcumin mediates anticancer effects by modulating multiple cell signaling pathways. *Clinical science* **2017**, *131* (15), 1781–1799.
- (8) Di Chio, C.; Previti, S.; Starvaggi, J.; De Luca, F.; Calabrò, M. L.; Zappalà, M.; Ettari, R. Drug Combination Studies of Isoquinolinone AM12 with Curcumin or Quercetin: A New Combination Strategy to Synergistically Inhibit 20S Proteasome. *International Journal of Molecular Sciences* **2024**, *25* (19), 10708.
- (9) Ebrahimzadeh, A.; Abbasi, F.; Ebrahimzadeh, A.; Jibril, A. T.; Milajerdi, A. Effects of curcumin supplementation on inflammatory biomarkers in patients with rheumatoid arthritis and ulcerative colitis: a systematic review and meta-analysis. *Complementary Therapies in Medicine* **2021**, *61*, No. 102773.
- (10) Baranauskas, V.; Jaruseviciene, R.; Grigalavicius, M.; Galgauskas, S.; Karabanovas, V.; Steponkiene, S. Biomimetic curcumin-loaded liposomes for the treatment of dry eyes and meibomian gland dysfunction: an in vivo study. *Journal of Clinical Medicine* **2024**, *13* (21), 6436.
- (11) Mahran, R. I.; Hagra, M. M.; Sun, D.; Brenner, D. E. Bringing curcumin to the clinic in cancer prevention: a review of strategies to enhance bioavailability and efficacy. *AAPS journal* **2017**, *19*, 54–81.
- (12) Omidian, H.; Wilson, R. L.; Chowdhury, S. D. Enhancing therapeutic efficacy of curcumin: Advances in delivery systems and clinical applications. *Gels* **2023**, *9* (8), 596.
- (13) Amekyeh, H.; Alkhader, E.; Sabra, R.; Billa, N. Prospects of curcumin nanoformulations in cancer management. *Molecules* **2022**, *27* (2), 361.
- (14) Wang, Y.; Sun, R.; Xu, X.; Du, M.; Zhu, B.; Wu, C. Mechanism of enhancing the water-solubility and stability of curcumin by using self-assembled cod protein nanoparticles at an alkaline pH. *Food & function* **2021**, *12* (24), 12696–12705.
- (15) Siviero, A.; Gallo, E.; Maggini, V.; Gori, L.; Mugelli, A.; Firenzuoli, F.; Vannacci, A. Curcumin, a golden spice with a low bioavailability. *Journal of Herbal Medicine* **2015**, *5* (2), 57–70.
- (16) Sahu, T.; Ratre, Y. K.; Chauhan, S.; Bhaskar, L.; Nair, M. P.; Verma, H. K. Nanotechnology based drug delivery system: Current strategies and emerging therapeutic potential for medical science. *Journal of Drug Delivery Science and Technology* **2021**, *63*, No. 102487.
- (17) Emeihe, E. V.; Nwankwo, E. I.; Ajegbile, M. D.; Olaboye, J. A.; Maha, C. C. Revolutionizing drug delivery systems: Nanotechnology-based approaches for targeted therapy. *Int. J. Life Sci. Res. Archive* **2024**, *7* (1), 040–058.
- (18) Liu, Y.; Liang, Y.; Yuhong, J.; Xin, P.; Han, J. L.; Du, Y.; Yu, X.; Zhu, R.; Zhang, M.; Chen, W.; Ma, Y. Advances in Nanotechnology for Enhancing the Solubility and Bioavailability of Poorly Soluble Drugs. *Drug Design Dev. Ther.* **2024**, 1469–1495.
- (19) Calixto, G. M. F.; Bernegossi, J.; De Freitas, L. M.; Fontana, C. R.; Chorilli, M. Nanotechnology-based drug delivery systems for photo-dynamic therapy of cancer: a review. *Molecules* **2016**, *21* (3), 342.
- (20) Bagade, O.; Sampathi, S. Restoration and Sustenance of Nano Drug Delivery Systems: Potential, Challenges, and Limitations. In *Biosystems, Biomedical & Drug Delivery Systems: Characterization, Restoration and Optimization*; Springer, 2024; pp 105–139.
- (21) Cao, J.; Gao, X.; Cheng, M.; Niu, X.; Li, X.; Zhang, Y.; Liu, Y.; Wang, W.; Yuan, Z. Reversible shielding between dual ligands for enhanced tumor accumulation of ZnPc-loaded micelles. *Nano Lett.* **2019**, *19* (3), 1665–1674.
- (22) Sun, X.; Wang, G.; Zhang, H.; Hu, S.; Liu, X.; Tang, J.; Shen, Y. The blood clearance kinetics and pathway of polymeric micelles in cancer drug delivery. *ACS Nano* **2018**, *12* (6), 6179–6192.
- (23) Xiang, L.; Wang, X.; Jiao, Q.; Shao, Y.; Luo, R.; Zhang, J.; Zheng, X.; Zhou, S.; Chen, Y. Selective inhibition of glycolysis in hepatic stellate cells and suppression of liver fibrogenesis with vitamin A-derivative decorated camptothecin micelles. *Acta biomaterialia* **2023**, *168*, 497–514.
- (24) Zhang, R.; Deng, H.; Lin, Y.; Wang, X.; He, B.; Dai, W.; Zhang, H.; Zheng, Y.; Zhang, Q.; Wang, X. A common strategy to improve transmembrane transport in polarized epithelial cells based on sorting signals: Guiding nanocarriers to TGN rather than to the basolateral plasma membrane directly. *J. Controlled Release* **2021**, *339*, 430–444.
- (25) Khang, M. K.; Zhou, J.; Co, C. M.; Li, S.; Tang, L. A pretargeting nanopatform for imaging and enhancing anti-inflammatory drug delivery. *Bioactive materials* **2020**, *5* (4), 1102–1112.
- (26) Schiliro, C.; Firestein, B. L. Mechanisms of metabolic reprogramming in cancer cells supporting enhanced growth and proliferation. *Cells* **2021**, *10* (5), 1056.
- (27) Ohshima, K.; Morii, E. Metabolic reprogramming of cancer cells during tumor progression and metastasis. *Metabolites* **2021**, *11* (1), 28.
- (28) Tang, Q.; Wu, S.; Zhao, B.; Li, Z.; Zhou, Q.; Yu, Y.; Yang, X.; Wang, R.; Wang, X.; Wu, W.; Wang, S. Reprogramming of glucose metabolism: the hallmark of malignant transformation and target for advanced diagnostics and treatments. *Biomed. Pharm.* **2024**, *178*, No. 117257.
- (29) Yoshida, G. J. Metabolic reprogramming: the emerging concept and associated therapeutic strategies. *J. Exp. Clin. Cancer Res.* **2015**, *34*, 1–10.
- (30) Pavlova, N. N.; Thompson, C. B. The emerging hallmarks of cancer metabolism. *Cell metabolism* **2016**, *23* (1), 27–47.
- (31) Chattopadhyay, C.; Oba, J.; Roszik, J.; Marszalek, J. R.; Chen, K.; Qi, Y.; Eterovic, K.; Robertson, A. G.; Burks, J. K.; McCannel, T. A.; Grimm, E. A.; Woodman, S. E. Elevated endogenous SDHA drives pathological metabolism in highly metastatic uveal melanoma. *Invest. Ophthalmol. Visual Sci.* **2019**, *60* (13), 4187–4195.
- (32) Dalla Pozza, E.; Dando, I.; Pacchiana, R.; Liboi, E.; Scupoli, M. T.; Donadelli, M.; Palmieri, M. Regulation of succinate dehydrogenase and role of succinate in cancer. In *Seminars in cell & developmental biology*, 2020; Elsevier: Vol. 98, pp 4–14.
- (33) Luo, Y.; Ma, J.; Lu, W. The significance of mitochondrial dysfunction in cancer. *International journal of molecular sciences* **2020**, *21* (16), 5598.
- (34) Etmianfar, M.; Sheykholeslami, S.; Khalili, V.; Mahdavi, S. Biocompatibility and drug delivery efficiency of PEG-b-PCL/hydroxyapatite bilayer coatings on Nitinol superelastic alloy. *Ceram. Int.* **2020**, *46* (8), 12711–12717.
- (35) Dethe, M. R.; A, P.; Ahmed, H.; Agrawal, M.; Roy, U.; Alexander, A. PCL-PEG copolymer based injectable thermosensitive hydrogels. *J. Controlled Release* **2022**, *343*, 217–236.
- (36) Liu, Z.; Smart, J. D.; Pannala, A. S. Recent developments in formulation design for improving oral bioavailability of curcumin: a review. *Journal of drug delivery science and technology* **2020**, *60*, No. 102082.
- (37) Aggarwal, B. B.; Sundaram, C.; Malani, N.; Ichikawa, H. Curcumin: the Indian solid gold. *molecular targets and therapeutic uses of curcumin in health and disease* **2007**, *595*, 1–75.
- (38) Liu, Z.; Lansley, A. B.; Duong, T. N.; Smart, J. D.; Pannala, A. S. Increasing cellular uptake and permeation of curcumin using a novel polymer-surfactant formulation. *Biomolecules* **2022**, *12* (12), 1739.
- (39) Zhu, Z.; Zhang, Y.; Wang, L.; Geng, H.; Li, M.; Chen, S.; Wang, X.; Chen, P.; Sun, C.; Zhang, C. Spatial Metabolomics Profiling Reveals Curcumin Induces Metabolic Reprogramming in Three-Dimensional Tumor Spheroids. *Metabolites* **2024**, *14* (9), 482.
- (40) Kujundžić, R. N.; Stepanić, V.; Milković, L.; Gašparović, A. Č.; Tomljanović, M.; Trošelj, K. G. Curcumin and its potential for systemic

targeting of inflamm-aging and metabolic reprogramming in cancer. *International journal of molecular sciences* **2019**, *20* (5), 1180.

(41) Bianchi, G.; Ravera, C.; Traverso, C.; Amaro, A.; Piaggio, F.; Emionite, L.; Bachetti, T.; Pfeffer, U.; Raffaghello, L. Curcumin induces a fatal energetic impairment in tumor cells in vitro and in vivo by inhibiting ATP-synthase activity. *Carcinogenesis* **2018**, *39* (9), 1141–1150.

(42) Wang, L.; Chen, X.; Du, Z.; Li, G.; Chen, M.; Chen, X.; Liang, G.; Chen, T. Curcumin suppresses gastric tumor cell growth via ROS-mediated DNA polymerase γ depletion disrupting cellular bioenergetics. *J. Exp. Clin. Cancer Res.* **2017**, *36*, 47.

(43) Schrimpe-Rutledge, A. C.; Codreanu, S. G.; Sherrod, S. D.; McLean, J. A. Untargeted metabolomics strategies—challenges and emerging directions. *J. Am. Soc. Mass Spectrom.* **2016**, *27* (12), 1897–1905.

(44) Wishart, D. S. Metabolomics for investigating physiological and pathophysiological processes. *Physiol. Rev.* **2019**, *99* (4), 1819–1875.

(45) Gowda, G. N.; Djukovic, D. Overview of mass spectrometry-based metabolomics: opportunities and challenges. *Mass Spectrometry in Metabolomics: Methods and Protocols* **2014**, 1198, 3–12.

(46) Jolliffe, I. T.; Cadima, J. Principal component analysis: a review and recent developments. *Philosophical transactions of the royal society A: Mathematical, Physical and Engineering Sciences* **2016**, *374* (2065), 20150202.

(47) Bylesjö, M.; Rantalainen, M.; Cloarec, O.; Nicholson, J. K.; Holmes, E.; Trygg, J. OPLS discriminant analysis: combining the strengths of PLS-DA and SIMCA classification. *Journal of Chemometrics: A Journal of the Chemometrics Society* **2006**, *20* (8–10), 341–351.

(48) Xiao, J. F.; Zhou, B.; Ransom, H. W. Metabolite identification and quantitation in LC-MS/MS-based metabolomics. *Trends in analytical chemistry: TRAC* **2012**, *32*, 1–14.

(49) Wang, M.; Pang, Y.; Guo, Y.; Tian, L.; Liu, Y.; Shen, C.; Liu, M.; Meng, Y.; Cai, Z.; Wang, Y.; Zhao, W. Metabolic reprogramming: A novel therapeutic target in diabetic kidney disease. *Front. Pharmacol.* **2022**, *13*, No. 970601.

(50) Cazzaniga, M.; Bonanni, B. Relationship between metabolic reprogramming and mitochondrial activity in cancer cells. Understanding the anticancer effect of metformin and its clinical implications. *Anticancer Res.* **2015**, *35* (11), 5789–5796.

(51) Sica, V.; Bravo-San Pedro, J. M.; Stoll, G.; Kroemer, G. Oxidative phosphorylation as a potential therapeutic target for cancer therapy. *International journal of cancer* **2020**, *146* (1), 10–17.

(52) Kalyanaraman, B.; Cheng, G.; Hardy, M.; Ouari, O.; Lopez, M.; Joseph, J.; Zielonka, J.; Dwinell, M. B. A review of the basics of mitochondrial bioenergetics, metabolism, and related signaling pathways in cancer cells: Therapeutic targeting of tumor mitochondria with lipophilic cationic compounds. *Redox biology* **2018**, *14*, 316–327.

(53) Boreel, D. F.; Span, P. N.; Heskamp, S.; Adema, G. J.; Bussink, J. Targeting oxidative phosphorylation to increase the efficacy of radio- and immune-combination therapy. *Clin. Cancer Res.* **2021**, *27* (11), 2970–2978.

(54) Sainero-Alcolado, L.; Liaño-Pons, J.; Ruiz-Pérez, M. V.; Arsenian-Henriksson, M. Targeting mitochondrial metabolism for precision medicine in cancer. *Cell Death & Differentiation* **2022**, *29* (7), 1304–1317.

(55) Guo, W.; Ma, J.; Yang, Y.; Guo, S.; Zhang, W.; Zhao, T.; Yi, X.; Wang, H.; Wang, S.; Liu, Y.; Dai, W.; Chen, X.; Shi, Q.; Wang, G.; Gao, T.; Li, C. ATP-citrate lyase epigenetically potentiates oxidative phosphorylation to promote melanoma growth and adaptive resistance to MAPK inhibition. *Clin. Cancer Res.* **2020**, *26* (11), 2725–2739.

(56) Duarte, T. T.; Spencer, C. T. Personalized proteomics: the future of precision medicine. *Proteomes* **2016**, *4* (4), 29.

(57) Li, L.; Shen, L.; Wu, H.; Li, M.; Chen, L.; Zhou, Q.; Ma, J.; Huai, C.; Zhou, W.; Wei, M.; Zhao, M.; Zhao, X.; Du, H.; Jiang, B.; Sun, Y.; Zhang, N.; Qin, S.; Xing, T. An integrated analysis identifies six molecular subtypes of pancreatic ductal adenocarcinoma revealing cellular and molecular landscape. *Carcinogenesis* **2023**, *44* (10–11), 726–740.

(58) Iannelli, F.; Lombardi, R.; Costantini, S.; Roca, M. S.; Addi, L.; Bruzzese, F.; Di Gennaro, E.; Budillon, A.; Pucci, B. Integrated proteomics and metabolomics analyses reveal new insights into the antitumor effects of valproic acid plus simvastatin combination in a prostate cancer xenograft model associated with downmodulation of YAP/TAZ signaling. *Cancer cell Int.* **2024**, *24* (1), 381.

(59) Esteban-Amo, M. J.; Jiménez-Cuadrado, P.; Serrano-Lorenzo, P.; de la Fuente, M. Á.; Simarro, M. Succinate Dehydrogenase and Human Disease: Novel Insights into a Well-Known Enzyme. *Biomedicines* **2024**, *12* (9), 2050.

(60) Rutter, J.; Winge, D. R.; Schiffman, J. D. Succinate dehydrogenase—assembly, regulation and role in human disease. *Mitochondrion* **2010**, *10* (4), 393–401.

(61) Wang, L.; Cybula, M.; Rostworowska, M.; Wang, L.; Mucha, P.; Bulicz, M.; Bieniasz, M. Upregulation of Succinate Dehydrogenase (SDHA) Contributes to Enhanced Bioenergetics of Ovarian Cancer Cells and Higher Sensitivity to Anti-Metabolic Agent Shikonin. *Cancers* **2022**, *14* (20), 5097.

(62) Markevich, N. I.; Markevich, L. N. Mathematical Modeling of ROS Production and Diode-like Behavior in the SDHA/SDHB Subcomplex of Succinate Dehydrogenases in Reverse Quinol-Fumarate Reductase Direction. *International Journal of Molecular Sciences* **2022**, *23* (24), 15596.

(63) Davidoff, D. F.; Luxford, C.; Kim, E.; Novos, T.; Horvath, A. R.; Gill, A. J.; Dwight, T.; Clifton-Bligh, R. J.; Burgess, J. R. Measuring Tumor Succinate and Fumarate to Resolve Pathogenicity of an SDHA Variant. *Clinical Chemistry* **2021**, *67* (4), 696–699.

(64) Elefantova, K.; Lakatos, B.; Kubickova, J.; Sulova, Z.; Breier, A. Detection of the mitochondrial membrane potential by the cationic dye JC-1 in L1210 cells with massive overexpression of the plasma membrane ABCB1 drug transporter. *International journal of molecular sciences* **2018**, *19* (7), 1985.

(65) Liesa, M.; Shrihari, O. S. Mitochondrial dynamics in the regulation of nutrient utilization and energy expenditure. *Cell metabolism* **2013**, *17* (4), 491–506.

(66) Bhatti, J. S.; Bhatti, G. K.; Reddy, P. H. Mitochondrial dysfunction and oxidative stress in metabolic disorders—A step towards mitochondria based therapeutic strategies. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* **2017**, *1863* (5), 1066–1077.

(67) Behl, A.; Solanki, S.; Paswan, S. K.; Datta, T. K.; Saini, A. K.; Saini, R. V.; Parmar, V. S.; Thakur, V. K.; Malhotra, S.; Chhillar, A. K. Biodegradable PEG-PCL nanoparticles for co-delivery of MUC1 inhibitor and doxorubicin for the confinement of triple-negative breast cancer. *Journal of Polymers and the Environment* **2023**, *31* (3), 999–1018.

(68) Grossen, P.; Witzigmann, D.; Sieber, S.; Huwyler, J. PEG-PCL-based nanomedicines: A biodegradable drug delivery system and its application. *J. Controlled Release* **2017**, *260*, 46–60.

(69) Ferrentino, N.; Behrooz Kohlan, T.; Mehrtashfar, S.; Finne-Wistrand, A.; Pappalardo, D. Dual-Responsive Nanoparticles for Smart Drug Delivery: A NIR Light-Sensitive and Redox-Reactive PEG-PCL-Based System. *Biomacromolecules* **2024**, *25* (12), 7660–7673.

(70) Jung, Y. J.; Kim, K. C.; Heo, J. Y.; Jing, K.; Lee, K. E.; Hwang, J. S.; Lim, K.; Jo, D. Y.; Ahn, J. P.; Kim, J. M.; Huh, K. M.; Park, J. I. Induction of angiogenesis by matrigel coating of VEGF-loaded PEG/PCL-based hydrogel scaffolds for hbmsc transplantation. *Mol. Cells* **2015**, *38* (7), 663–668.