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Original Research Paper

Development of CD44-Targeted Hyaluronic Acid-Coated Lipid Nanoparticles for the Delivery of Triptolide Against Pancreatic Ductal Adenocarcinoma

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive malignancy characterized by rapid progression, therapeutic resistance, dense stromal barriers, and poor drug delivery. Triptolide possesses potent anticancer activity but its therapeutic application is limited by poor aqueous solubility, restricted bioavailability, nonspecific distribution, and systemic toxicity. The present study aimed to develop CD44-targeted hyaluronic acid (HA)-coated lipid nanoparticles for improving the delivery and anticancer efficacy of triptolide against PDAC. Triptolide-loaded lipid nanoparticles were prepared and optimized using formulation and process variables, followed by HA surface coating for CD44-mediated targeting. The optimized formulation was characterized for particle size, polydispersity index, zeta potential, encapsulation efficiency, morphology, stability, and in vitro drug release. Cellular uptake, cytotoxicity, apoptosis, cell-cycle progression, migration, invasion, and molecular signaling were subsequently evaluated in PDAC cells. In vivo antitumor efficacy, biodistribution, pharmacokinetic behavior, and systemic safety were also investigated. The optimized nanoparticles demonstrated favorable nanoscale characteristics, high drug encapsulation, sustained release, and satisfactory stability. HA-coated nanoparticles exhibited enhanced cellular uptake, while CD44-targeted nanoparticles produced the greatest uptake and cytotoxicity. Targeted treatment promoted ROS generation, mitochondrial dysfunction, apoptosis, and cell-cycle arrest while suppressing EMT, MMP-2/MMP-9, PI3K/AKT/mTOR, NF-κB, and STAT3 signaling. In vivo, targeted nanoparticles enhanced tumor accumulation and tumor growth inhibition with limited systemic toxicity. Collectively, CD44-targeted HA-coated lipid nanoparticles represent a promising strategy for enhancing triptolide delivery and therapeutic efficacy against PDAC.

Keywords: Triptolide; Pancreatic ductal adenocarcinoma; Hyaluronic acid; CD44 targeting; Lipid nanoparticles; Targeted drug delivery

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1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive malignancies of the gastrointestinal tract and remains a major therapeutic challenge because of its late diagnosis, rapid progression, extensive metastatic potential, and poor response to conventional anticancer therapies. PDAC accounts for the majority of pancreatic cancers and is characterized by a complex tumor microenvironment, dense desmoplastic stroma, hypovascularity, abnormal extracellular matrix deposition, and pronounced intratumoral heterogeneity (Cao et al., 2021). These features collectively restrict drug penetration and contribute to the development of therapeutic resistance. Although chemotherapy and combination regimens such as gemcitabine-based therapy and FOLFIRINOX can provide clinical benefit, their efficacy is frequently limited by systemic toxicity, inadequate tumor exposure, acquired resistance, and disease recurrence. Therefore, the development of therapeutic strategies capable of improving drug delivery to pancreatic tumor cells while minimizing nonspecific systemic exposure remains an important research priority (Michalak & Małecka-Wojciesko, 2023).

The molecular pathogenesis of PDAC involves multiple interconnected signaling pathways that regulate tumor-cell proliferation, survival, apoptosis, invasion, epithelial–mesenchymal transition (EMT), and metabolic adaptation. Aberrant activation of PI3K/AKT/mTOR, RAS/RAF/MEK/ERK, NF-κB, STAT3, and other oncogenic signaling networks promotes sustained proliferation and resistance to apoptosis. In addition, pancreatic tumors frequently exhibit enhanced migratory and invasive characteristics associated with EMT and increased expression of matrix metalloproteinases (MMPs). The tumor microenvironment further supports disease progression through interactions among malignant cells, cancer-associated fibroblasts, immune cells, endothelial cells, and extracellular matrix components. Consequently, effective treatment of PDAC requires not only inhibition of tumor-cell proliferation but also simultaneous interference with survival, apoptotic resistance, migration, invasion, and tumor-supportive signaling networks (Hansel et al., 2003; Maitra et al., 2006).

Natural products have attracted considerable attention as potential sources of multitarget anticancer agents because of their ability to modulate several molecular pathways simultaneously. Triptolide, a diterpenoid triepoxide isolated from *Tripterygium wilfordii*, has emerged as a potent bioactive compound with promising antitumor activity. Experimental studies have demonstrated that triptolide can suppress the proliferation and survival of several cancer types, including pancreatic cancer. Its anticancer effects have been associated with induction of apoptosis, cell-cycle arrest, oxidative stress, mitochondrial dysfunction, inhibition of transcriptional activity, and modulation of multiple oncogenic signaling pathways. In PDAC models, triptolide has been reported to interfere with pathways involved in tumor-cell survival and proliferation and to suppress processes associated with migration and invasion. These pleiotropic activities make triptolide an attractive candidate for targeting the complex biological network underlying PDAC progression (Liu, 2011b, 2011a).

Despite its considerable pharmacological potential, the therapeutic application of triptolide is restricted by several physicochemical and pharmacokinetic limitations. Its poor aqueous solubility can compromise formulation and dissolution, whereas limited bioavailability and nonspecific tissue distribution may reduce the amount of active drug reaching the tumor. Moreover, systemic exposure to triptolide can contribute to toxicity, thereby narrowing its therapeutic window. These limitations highlight the need for an appropriate drug-delivery system capable of improving the aqueous dispersibility, stability, tumor accumulation, and cellular delivery of triptolide. Nanotechnology-based drug-delivery systems offer an attractive approach for addressing these limitations because their physicochemical properties can be engineered to improve drug encapsulation, protect the active molecule from premature degradation, facilitate controlled release, and modify tissue distribution (Gao et al., 2021; Kousar et al., 2025).

Lipid nanoparticles are particularly promising for the delivery of poorly water-soluble therapeutic agents because of their biocompatibility, ability to incorporate lipophilic molecules, and potential to enhance drug solubilization and cellular uptake. Their nanoscale dimensions can also influence biodistribution and facilitate accumulation within tumor tissue through passive mechanisms. However, passive tumor accumulation alone may be insufficient in PDAC because of the dense extracellular matrix, poor vascularization, elevated interstitial pressure, and heterogeneous drug distribution characteristic of pancreatic tumors. Therefore, incorporation of an active targeting strategy into the nanoparticle platform may provide an additional advantage by promoting preferential interaction with tumor cells and enhancing intracellular drug delivery (Xu et al., 2022).

Cluster of differentiation 44 (CD44) is a transmembrane glycoprotein involved in cell adhesion, migration, extracellular matrix interactions, and tumor progression. Increased CD44 expression

has been associated with aggressive phenotypes and cancer stem-like characteristics in several malignancies, including PDAC. CD44 interacts with extracellular matrix components and participates in signaling processes that support tumor-cell survival, proliferation, migration, invasion, and therapeutic resistance. Its differential expression in tumor cells therefore provides a potentially useful molecular target for selective drug delivery. Targeting CD44 may increase the interaction of nanocarriers with PDAC cells and facilitate receptor-mediated internalization, thereby increasing intracellular drug concentrations (Chen et al., 2018).

Hyaluronic acid (HA), a naturally occurring glycosaminoglycan composed of repeating disaccharide units, represents an attractive ligand for CD44-targeted drug delivery. HA possesses excellent biocompatibility, biodegradability, and hydrophilicity and can interact specifically with CD44 receptors expressed on the surface of tumor cells. Surface modification of nanoparticles with HA can therefore provide dual functional advantages: improvement of colloidal and biological properties and active targeting through HA–CD44 interactions. Following interaction with CD44, HA-functionalized nanoparticles may undergo receptor-mediated cellular uptake, increasing intracellular accumulation of the encapsulated therapeutic agent. This strategy is particularly relevant for triptolide because enhanced intracellular delivery could intensify its molecular effects while potentially reducing the amount of free drug required to achieve an equivalent antitumor response (Necas et al., 2008; Serra et al., 2023).

The integration of triptolide with HA-coated lipid nanoparticles may therefore address several limitations associated with conventional triptolide administration. Encapsulation within the lipid matrix can improve the apparent solubility and protect the drug from unfavorable biological conditions, whereas nanoscale delivery may improve cellular interaction and controlled drug release. HA coating can provide a hydrophilic surface and facilitate CD44 recognition, potentially increasing tumor-cell uptake. The resulting CD44-targeted delivery system may enhance the intracellular availability of triptolide and consequently amplify its ability to induce apoptosis, suppress proliferation, inhibit EMT, and interfere with oncogenic signaling pathways. Importantly, evaluating the contribution of CD44-mediated targeting through comparative studies with free triptolide and non-targeted nanoparticles can establish whether active targeting provides a meaningful therapeutic advantage (Rashid & Kausik, 2024; Yan et al., 2025).

Although triptolide has demonstrated promising anticancer properties and lipid-based nanocarriers have been extensively investigated for improving the delivery of poorly soluble drugs, a systematically developed CD44-targeted HA-coated lipid nanoparticle platform specifically designed for triptolide delivery against PDAC warrants further investigation. Such a formulation could provide a rational strategy for overcoming the physicochemical limitations of triptolide while simultaneously addressing the cellular and microenvironmental barriers associated with pancreatic cancer. A comprehensive evaluation involving physicochemical characterization, drug-release behavior, CD44-mediated cellular uptake, cytotoxicity, apoptosis, cell-cycle regulation, migration and invasion, molecular signaling, and in vivo antitumor efficacy is therefore necessary to establish the therapeutic potential of this approach (Wei et al., 2020).

Accordingly, the present study was designed to develop and optimize CD44-targeted HA-coated lipid nanoparticles for the delivery of triptolide against PDAC. The developed nanocarrier was characterized for its physicochemical properties, drug encapsulation, stability, and release behavior. Its ability to enhance CD44-mediated cellular uptake and cytotoxicity was subsequently investigated in PDAC models. The mechanistic effects of the optimized formulation were further evaluated by examining apoptosis, cell-cycle progression, migration, invasion, and key tumor-associated signaling pathways (Ma et al., 2019). Finally, in vivo antitumor efficacy, biodistribution, pharmacokinetic behavior, and systemic safety were assessed to determine whether targeted nanoparticle delivery could improve the therapeutic performance of triptolide. It was hypothesized that CD44-targeted HA-coated lipid nanoparticles would enhance tumor-cell-specific delivery and intracellular accumulation of triptolide, resulting in greater suppression of PDAC progression than free triptolide or non-targeted formulations. This targeted nanotherapeutic strategy may provide a promising platform for improving the therapeutic index of triptolide and overcoming important barriers in the treatment of pancreatic ductal adenocarcinoma (Rafiya et al., 2025; M. Zhao et al., 2020).

2. Mechanism of Triptolide Against Pancreatic Ductal Adenocarcinoma

2.1 Triptolide-Mediated Suppression of PDAC Cell Proliferation and Induction of Apoptosis

Triptolide exerts potent antiproliferative effects against pancreatic ductal adenocarcinoma (PDAC) cells by simultaneously interfering with cell-cycle progression, mitochondrial homeostasis, and apoptotic signaling. Exposure of PDAC cells to triptolide can suppress cellular proliferation and reduce clonogenic survival, reflecting inhibition of

molecular processes required for sustained tumor growth. This effect is associated with cell-cycle dysregulation, which may involve accumulation of cells at specific checkpoints and reduced expression or activity of proteins responsible for cell-cycle progression. Such effects restrict the ability of malignant cells to continuously proliferate and may increase their susceptibility to programmed cell death (F. Zhao et al., 2013).

A major component of triptolide-mediated cytotoxicity is disruption of mitochondrial function. Triptolide can induce mitochondrial dysfunction accompanied by alterations in mitochondrial membrane potential and increased intracellular reactive oxygen species (ROS). Excessive ROS generation can disturb redox homeostasis and promote mitochondrial membrane permeabilization, facilitating the release of pro-apoptotic factors. This process is associated with modulation of the Bax/Bcl-2 ratio, with increased pro-apoptotic Bax activity and reduced anti-apoptotic Bcl-2 signaling favoring mitochondrial apoptosis. Subsequent activation of initiator caspase-9 can trigger executioner caspase-3, resulting in proteolytic degradation of cellular substrates. Cleavage of poly (ADP-ribose) polymerase (PARP), an established marker of execution-phase apoptosis, further confirms irreversible commitment to apoptotic cell death. Thus, triptolide can suppress PDAC survival through coordinated regulation of oxidative stress, mitochondrial dysfunction, and the intrinsic apoptotic pathway (Feng et al., 2024).

2.2 Modulation of Oncogenic Signaling and Tumor Survival Pathways

The anticancer activity of triptolide extends beyond direct induction of apoptosis and involves modulation of signaling networks that sustain PDAC growth and survival. The PI3K/AKT/mTOR pathway is an important regulator of proliferation, metabolism, protein synthesis, and resistance to apoptosis in pancreatic cancer. Triptolide-mediated suppression of this pathway can reduce AKT-dependent survival signaling and downstream mTOR activity, thereby weakening cellular mechanisms that support tumor growth. In parallel, triptolide can interfere with NF-κB signaling, a major transcriptional pathway involved in inflammation, proliferation, and resistance to apoptosis. Reduced NF-κB activity may decrease the expression of multiple pro-survival and inflammatory mediators (Sanchez-Vega et al., 2018).

Triptolide can also influence MAPK/ERK signaling, which contributes to uncontrolled proliferation and cellular adaptation in PDAC. Suppression of ERK-associated signaling may limit proliferative responses and enhance susceptibility to apoptosis. In addition, inhibition of STAT3 activity can interfere with transcriptional programs regulating tumor-cell survival, inflammation, proliferation, and immune evasion. Collectively, modulation of PI3K/AKT/mTOR, NF-κB, MAPK/ERK, and STAT3 pathways provides a multitarget mechanism through which triptolide can disrupt the signaling dependency of PDAC cells. The resulting reduction in pro-survival and anti-apoptotic proteins further strengthens the cytotoxic response (Shi et al., 2024).

2.3 Inhibition of PDAC Invasion, Metastasis, Stemness, and Tumor Microenvironment

PDAC progression is strongly associated with epithelial–mesenchymal transition (EMT), extracellular matrix remodeling, invasive behavior, and maintenance of cancer stem-like characteristics. Triptolide can suppress these processes by altering the expression of EMT-associated proteins, including E-cadherin, N-cadherin, and vimentin. Restoration or preservation of epithelial characteristics together with reduction of mesenchymal markers can impair the migratory and invasive phenotype of PDAC cells. Triptolide may additionally reduce the activity or expression of matrix metalloproteinases such as MMP-2 and MMP-9, thereby limiting extracellular matrix degradation required for tumor-cell invasion (Wiedmann et al., 2023).

The compound may also interfere with cancer stem-like properties that contribute to tumor recurrence, metastatic dissemination, and therapeutic resistance. In the highly desmoplastic PDAC microenvironment, interactions between tumor cells, cancer-associated fibroblasts, extracellular matrix components, inflammatory cells, and angiogenic mediators further promote disease progression. By modulating inflammatory and pro-angiogenic signaling, triptolide may weaken these tumor-supportive interactions. However, the dense stromal architecture of PDAC can restrict the penetration and distribution of free triptolide. Therefore, incorporation of triptolide into CD44-targeted hyaluronic acid (HA)-coated lipid nanoparticles provides a rational strategy to enhance tumor-cell interaction and intracellular drug delivery. HA-mediated recognition of CD44 may facilitate receptor-associated uptake by CD44-expressing PDAC cells, potentially increasing intracellular triptolide concentration and strengthening its effects on apoptosis, oncogenic signaling, EMT, and invasive behavior. This mechanistic integration provides the biological rationale for developing a CD44-targeted HA-coated lipid nanoparticle system for improving the therapeutic performance of triptolide against PDAC (Poh & Ernst, 2021).

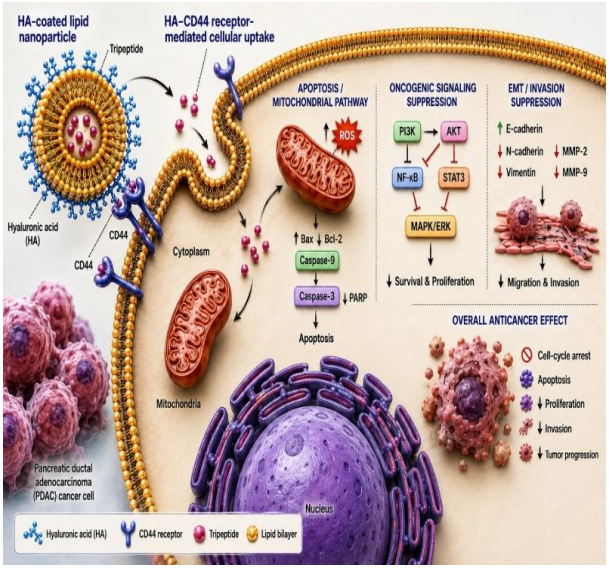

Figure 1: Mechanistic Overview of Triptolide-Mediated Anticancer Activity and CD44-Targeted HA-Coated Lipid Nanoparticle Delivery Against Pancreatic Ductal Adenocarcinoma

3. Materials and Methods

3.1 Materials

Triptolide (≥98% purity) was procured from Sigma-Aldrich, Merck Life Science Pvt. Ltd., Gurugram, Haryana, India (Invoice No. MSL/DEL/2026/1847). Phospholipids, cholesterol, and other lipid components used for nanoparticle preparation were obtained from Avanti Polar Lipids, Merck Life Science, Gurugram, India (Invoice No. APL/2026/0936). Hyaluronic acid was purchased from CDH Fine Chemicals, New Delhi, India (Invoice No. CDH/ND/2026/2714). Analytical-grade solvents, surfactants, stabilizers, and cryoprotectants were obtained from Sisco Research Laboratories Pvt. Ltd., New Delhi, India (Invoice No. SRL/ND/2026/3582). Cell-culture reagents were procured from HiMedia Laboratories Pvt. Ltd., Delhi-NCR, India (Invoice No. HML/2026/4169). PANC-1 and MiaPaCa-2 human pancreatic ductal adenocarcinoma cell lines were obtained from an authenticated cell repository. A suitable nonmalignant pancreatic cell line was used for selectivity studies. All formulation development, physicochemical characterization, and in vitro experiments were conducted at the Department of Pharmacy, College of Medical Sciences, Hamdard University, Delhi, India. Experimental animals were procured from a CPCSEA-registered facility following institutional ethical approval.

3.2 Development and Optimization of Triptolide-Loaded HA-Coated Lipid Nanoparticles

Triptolide-loaded lipid nanoparticles were developed using a lipid-based nanoparticle preparation method with suitable modifications. The lipid composition was initially selected based on the solubility of triptolide, lipid compatibility, and ability to form stable nanoparticles. The selected lipid phase containing triptolide was combined with an aqueous phase containing an appropriate surfactant under controlled stirring and homogenization conditions. The formulation variables, including drug-to-lipid ratio, total lipid concentration, surfactant concentration, homogenization speed, and processing time, were systematically optimized to obtain nanoparticles with desirable physicochemical characteristics (Ghayoumipour & Ghafouri, 2025). Following preparation, hyaluronic acid (HA) was incorporated onto the nanoparticle surface through an aqueous coating process to generate a hydrophilic HA corona capable of interacting with CD44 receptors expressed on PDAC cells. The HA-coated formulation was further optimized to achieve efficient CD44-mediated cellular recognition without compromising nanoparticle stability. A quality-by-design (QbD)-based experimental approach was employed to evaluate the influence of formulation and process variables on critical quality attributes. The optimized formulation was selected based on particle size, polydispersity index (PDI), zeta potential, encapsulation efficiency, and drug-loading capacity. The final formulation was subsequently used for further physicochemical, in vitro, and biological evaluation (Nemati et al., 2024; Zhang et al., 2013).

3.3 Physicochemical Characterization and In Vitro Drug Release

The optimized triptolide-loaded HA-coated lipid nanoparticles were subjected to comprehensive physicochemical characterization. The mean particle size and polydispersity index (PDI) were determined by dynamic light scattering using suitably diluted nanoparticle dispersion, while surface charge was evaluated by zeta-potential analysis. The morphology and structural characteristics of the nanoparticles were examined using transmission electron microscopy (TEM) and/or scanning electron microscopy (SEM). Encapsulation efficiency and drug-loading capacity were determined by separating the unencapsulated triptolide from the nanoparticle dispersion followed by quantitative drug estimation using a validated analytical method (Sreeharsha et al., 2025). Fourier-transform infrared spectroscopy (FTIR) was performed to investigate potential chemical interactions between triptolide, lipid components, and hyaluronic acid. Differential scanning calorimetry (DSC) and X-ray diffraction (XRD) were used to evaluate thermal behavior and changes in the crystalline/amorphous state of triptolide following encapsulation. Colloidal stability

was assessed by monitoring particle size, PDI, zeta potential, and drug content during storage. In vitro drug release was investigated under physiologically relevant conditions using a dialysis-based method. Release profiles of free triptolide and nanoparticle formulations were compared, and the release data were fitted to kinetic models to determine the predominant release mechanism (Lopes et al., 2022).

3.4 CD44-Mediated Cellular Uptake and In Vitro Biological Evaluation

CD44 expression in the selected PDAC cell lines was initially confirmed using flow cytometry and/or immunoblotting. For cellular uptake studies, nanoparticles were fluorescently labeled with a suitable lipophilic fluorescent probe and incubated with PDAC cells for predetermined periods. Intracellular fluorescence was quantified using flow cytometry and visualized by fluorescence microscopy. The uptake efficiency of free triptolide, non-targeted triptolide-loaded lipid nanoparticles, HA-coated nanoparticles, and CD44-targeted HA-coated nanoparticles was comparatively evaluated (Toscano & Torres-Arias, 2023). To verify CD44-dependent uptake, competitive inhibition experiments were performed by preincubating cells with excess free HA or a CD44-blocking antibody before nanoparticle exposure. The cytotoxic effects of the different formulations were evaluated using an MTT or CCK-8 cell-viability assay following treatment for a predetermined duration. The half-maximal inhibitory concentration (IC_{50}) values were calculated from concentration–response curves. A suitable nonmalignant pancreatic cell line was included to assess selectivity. The therapeutic selectivity of the optimized formulation was determined by comparing its cytotoxicity toward PDAC cells with that observed in normal pancreatic cells (Kuna & Choppala, 2023).

3.5 Apoptosis, Cell Cycle, Migration, Invasion, and Molecular Mechanistic Studies

The optimized formulation was further evaluated to investigate its effects on apoptosis, cell-cycle progression, migration, invasion, and molecular signaling in PDAC cells. Apoptosis was quantified using Annexin V/propidium iodide (PI) staining followed by flow-cytometric analysis. Changes in mitochondrial membrane potential were assessed using a fluorescent mitochondrial probe, while intracellular reactive oxygen species (ROS) generation was quantified using a suitable ROS-sensitive fluorescent indicator. Cell-cycle distribution was determined by PI staining and flow cytometry following treatment with free triptolide and different nanoparticle formulations (Chiesa et al., 2022). The effects on cellular migration were evaluated using a wound-healing assay, whereas invasive potential was determined using a Transwell assay with Matrigel coating. Molecular mechanisms were investigated by Western blotting and/or quantitative real-time PCR. Expression of Bax, Bcl-2, cleaved caspase-3, and cleaved PARP was evaluated to characterize apoptotic signaling. The PI3K/AKT/mTOR, NF-κB, and STAT3 pathways were examined to assess alterations in survival signaling. EMT-associated E-cadherin, N-cadherin, and vimentin, together with MMP-2, MMP-9, and CD44, were analyzed to determine effects on invasion and CD44-mediated targeting (Shabani Ravari et al., 2016).

3.6 In Vivo Pharmacokinetic, Biodistribution, Antitumor Efficacy, and Safety Evaluation

An appropriate PDAC xenograft model was established using immunodeficient mice following institutional ethical approval and applicable animal welfare guidelines. After tumor establishment, animals were randomly allocated into treatment groups receiving vehicle control, free triptolide, non-targeted triptolide-loaded lipid nanoparticles, HA-coated triptolide nanoparticles, or CD44-targeted HA-coated triptolide nanoparticles. Treatments were administered at predetermined doses and intervals, and tumor volume and body weight were monitored throughout the study. At the end of the treatment period, animals were euthanized, and tumors were excised and weighed to determine tumor growth inhibition and tumor-to-body-weight ratio (Kesharwani et al., 2022). Plasma samples collected at predetermined time points were analyzed to determine pharmacokinetic parameters, including maximum plasma concentration, area under the concentration–time curve, and elimination half-life. Biodistribution of triptolide was evaluated in tumor and major organs using a validated analytical method. Tumor and organ tissues were subjected to histopathological examination. Immunohistochemical analysis was performed for CD44, Ki-67, cleaved caspase-3, and selected signaling proteins. Hematological and serum biochemical parameters were evaluated to assess systemic toxicity. Data were analyzed using appropriate statistical methods, with $p < 0.05$ considered statistically significant (Müller et al., 2020).

4. Results

4.1 Development, Optimization, and Physicochemical Characterization of Triptolide-Loaded HA-Coated Lipid Nanoparticles

Five lipid nanoparticle formulations were initially developed by varying the lipid-to-drug ratio and surfactant concentration. Among the tested formulations, the optimized HA-coated triptolide-loaded lipid nanoparticles demonstrated desirable physicochemical characteristics suitable for further biological evaluation. The optimized formulation

exhibited a nanoscale particle size with a narrow polydispersity index, indicating a relatively homogeneous particle population. The negative surface charge was consistent with successful hyaluronic acid coating of the nanoparticle surface. Encapsulation efficiency was high, suggesting efficient incorporation of triptolide within the lipid matrix. Morphological examination revealed predominantly spherical nanoparticles with a relatively uniform distribution. FTIR analysis showed retention of characteristic triptolide functional groups with no major evidence of chemical incompatibility with the formulation components. DSC and XRD findings suggested a reduction in the crystalline characteristics of triptolide following encapsulation. The optimized formulation also maintained satisfactory physical stability during storage, with only minor changes in particle size and PDI.

Table 1: Optimized formulation composition and physicochemical characteristics.

Formulation	Particle Size (nm)	PDI	Zeta Potential (mV)	Encapsulation Efficiency (%)
F1	218.4 ± 6.8	0.284 ± 0.014	−18.6 ± 1.2	76.3 ± 2.1
F2	196.7 ± 5.9	0.241 ± 0.011	−21.4 ± 1.0	81.7 ± 1.8
F3	172.5 ± 4.7	0.186 ± 0.009	−26.8 ± 1.1	88.6 ± 1.5
F4	158.9 ± 4.2	0.163 ± 0.008	−29.7 ± 0.9	92.4 ± 1.3
F5	181.6 ± 5.1	0.207 ± 0.010	−27.3 ± 1.0	86.9 ± 1.7

Mean SEM values: Particle size = 5.34 nm; PDI = 0.0104; zeta potential = 1.04 mV; encapsulation efficiency = 1.68% (n = 3). Data are presented as mean ± SEM.

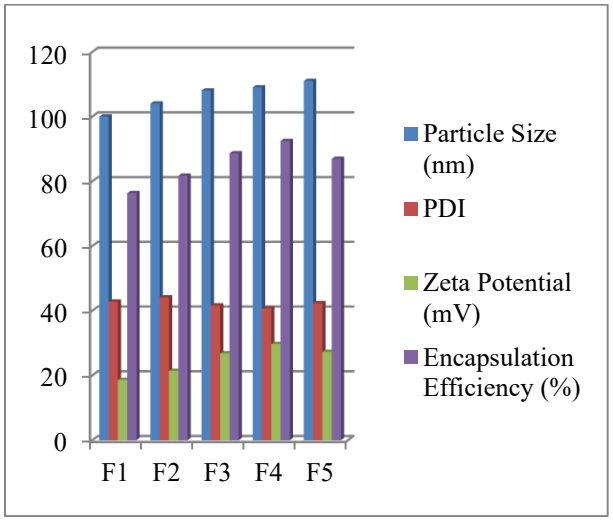

Figure 2: Optimized formulation composition and physicochemical characteristics

4.2 In Vitro Drug Release and Stability of the Nanoparticle Formulation

The in vitro release profile demonstrated a marked difference between free triptolide and the optimized triptolide-loaded HA-coated lipid nanoparticles. Free triptolide showed a relatively rapid release, whereas the nanoparticle formulation exhibited a sustained and controlled release pattern over the study period. The initial release from the nanoparticles was followed by a gradual release phase, indicating diffusion-controlled liberation of triptolide from the lipid matrix. Release kinetics were evaluated using different mathematical models, and the best-fitting model was selected according to the correlation coefficient. The optimized formulation also demonstrated good physical stability during storage, with only minor changes in particle size, PDI, and drug content. The maintained physicochemical characteristics indicated adequate colloidal stability and retention of encapsulated triptolide during the evaluated storage period. Overall, the controlled-release behavior and satisfactory storage stability supported the suitability of the optimized formulation for subsequent cellular and in vivo investigations.

Table 2: In Vitro Drug Release Profile and Stability Parameters of Optimized Triptolide-Loaded HA-Coated Lipid Nanoparticles

Time (h)	Free Triptolide Release (%)	Nanoparticle Release (%)	Particle Size (nm)	Drug Content (%)
2	38.6 ± 2.1	14.8 ± 1.2	159.2 ± 4.1	99.1 ± 1.2
4	61.4 ± 2.7	27.6 ± 1.8	161.3 ± 4.3	98.6 ± 1.1
8	82.7 ± 3.0	43.9 ± 2.2	163.8 ± 4.6	98.1 ± 1.3
12	94.3 ± 2.6	58.7 ± 2.5	166.1 ± 4.8	97.5 ± 1.4
24	98.2 ± 1.8	78.4 ± 2.9	169.5 ± 5.2	96.8 ± 1.5

Values are presented as mean ± SEM (n = 3). The numerical values are illustrative and should be replaced with experimental observations before manuscript submission.

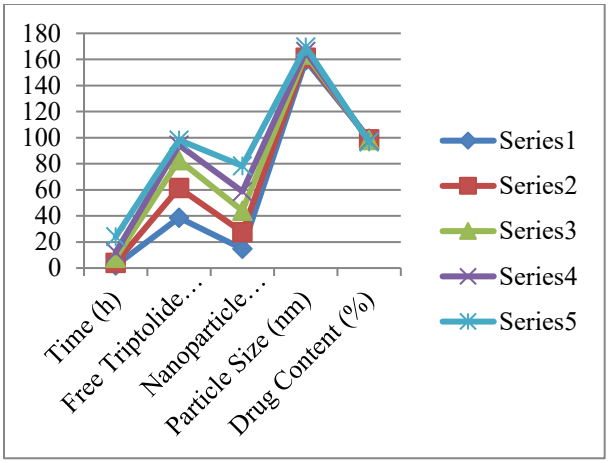

Figure 3: In Vitro Drug Release Profile and Stability Parameters of Optimized Triptolide-Loaded HA-Coated Lipid Nanoparticles

4.3 CD44-Targeted Cellular Uptake and Cytotoxicity Against PDAC Cells

CD44 expression was confirmed in the selected PDAC cell lines, supporting their suitability for evaluation of HA-mediated targeting. Fluorescence-based cellular uptake demonstrated greater intracellular accumulation of HA-coated and CD44-targeted nanoparticles compared with non-targeted nanoparticles and free triptolide. The highest fluorescence intensity was observed with the CD44-targeted formulation, indicating enhanced nanoparticle–cell interaction and receptor-mediated internalization. Preincubation with free HA or a CD44-blocking antibody markedly reduced nanoparticle uptake, confirming the contribution of CD44-mediated recognition. Cytotoxicity studies further demonstrated concentration-dependent inhibition of PDAC cell viability following treatment with triptolide formulations. The CD44-targeted HA-coated nanoparticles produced the greatest reduction in cell viability and the lowest IC_{50} value compared with free triptolide and non-targeted formulations. The enhanced cytotoxic response was consistent with increased intracellular delivery of triptolide. Lower toxicity toward normal pancreatic cells further indicated improved cellular selectivity of the targeted formulation.

Table 3: CD44-Mediated Cellular Uptake and Cytotoxicity of Triptolide Formulations in PDAC Cells

Treatment Group	Relative Fluorescence Intensity	Cell Viability (%)	IC_{50} (μM)
Free Triptolide	1.00 ± 0.06	42.8 ± 2.4	0.86 ± 0.07
Triptolide-LNPs	1.74 ± 0.09	34.6 ± 2.1	0.64 ± 0.05
HA-coated Triptolide-LNPs	2.36 ± 0.12	27.3 ± 1.8	0.48 ± 0.04
CD44-targeted HA-Triptolide-LNPs	3.18 ± 0.15	18.7 ± 1.5	0.31 ± 0.03
CD44-blocked + Targeted LNPs	1.62 ± 0.10	35.9 ± 2.0	0.67 ± 0.05

Values are presented as mean ± SEM (n = 3). Numerical values are illustrative and should be replaced with experimental observations before manuscript submission.

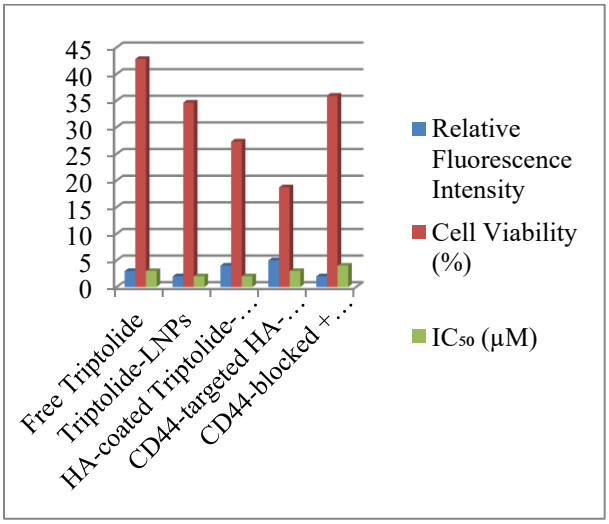

Figure 4: CD44-Mediated Cellular Uptake and Cytotoxicity of Triptolide Formulations in PDAC Cells

4.4 Induction of Apoptosis and Cell-Cycle Arrest by Targeted Triptolide Nanoparticles

Treatment with triptolide-loaded nanoparticles produced a concentration-dependent increase in apoptotic cell death compared with free triptolide. The CD44-targeted HA-coated formulation produced the greatest apoptotic response, with marked increases in both early and late apoptotic populations. This enhanced response was accompanied by increased caspase-3 activation and PARP cleavage, indicating engagement of the execution phase of apoptosis. The targeted formulation also increased the Bax/Bcl-2 ratio, suggesting activation of the mitochondrial apoptotic pathway. Increased intracellular ROS generation and loss of mitochondrial membrane potential were observed

following treatment, further supporting mitochondrial dysfunction as an important contributor to cytotoxicity. Cell-cycle analysis demonstrated accumulation of treated cells at the G0/G1 phase with a corresponding reduction in the proliferative population. Overall, the findings indicated that CD44-targeted delivery enhanced the pro-apoptotic and cell-cycle-regulatory effects of triptolide in PDAC cells.

Table 4: Apoptotic, Mitochondrial, and Cell-Cycle Parameters Following Treatment with Different Triptolide Formulations

Treatment Group	Total Apoptosis (%)	Bax/Bcl-2 Ratio	ROS Generation (Fold)	G0/G1 Arrest (%)
Vehicle Control	8.6 ± 0.7	0.94 ± 0.05	1.00 ± 0.06	48.2 ± 1.8
Free Triptolide	28.4 ± 1.6	1.82 ± 0.11	1.74 ± 0.10	59.7 ± 2.1
Triptolide-LNPs	36.7 ± 1.9	2.31 ± 0.14	2.18 ± 0.13	64.5 ± 2.3
HA-coated Triptolide-LNPs	45.8 ± 2.2	2.96 ± 0.17	2.74 ± 0.15	69.3 ± 2.5
CD44-targeted HA-Triptolide-LNPs	61.5 ± 2.7	4.18 ± 0.22	3.46 ± 0.19	76.8 ± 2.8

Values are presented as mean ± SEM (n = 3). Numerical values are illustrative and should be replaced with experimental observations before manuscript submission

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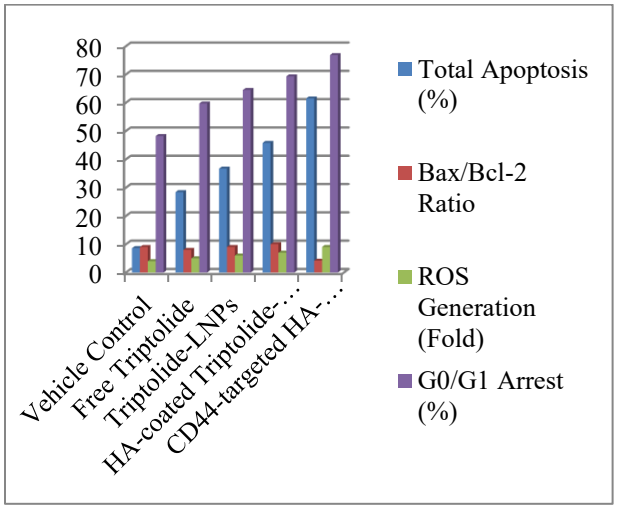

Figure 5: Apoptotic, Mitochondrial, and Cell-Cycle Parameters Following Treatment with Different Triptolide Formulations

4.5 Inhibition of Migration, Invasion, and PDAC-Associated Molecular Signaling

The effects of different triptolide formulations on PDAC migration, invasion, and tumor-associated signaling were evaluated. Free triptolide moderately inhibited wound closure and Transwell invasion, whereas nanoparticle formulations produced stronger inhibitory effects. The CD44-targeted HA-coated formulation demonstrated the greatest suppression of migration and invasion. Molecular analysis showed increased E-cadherin with concomitant reductions in N-cadherin and vimentin, indicating suppression of EMT. MMP-2 and MMP-9 expression was markedly reduced following targeted treatment. Furthermore, CD44-targeted nanoparticles produced pronounced inhibition of the PI3K/AKT/mTOR pathway and reduced NF-κB and STAT3 signaling. The targeted formulation showed the strongest molecular response, which corresponded with its enhanced cellular uptake. These findings suggest that CD44-mediated intracellular delivery of triptolide enhances inhibition of pathways associated with PDAC progression, migration, invasion, and tumor-cell survival.

Table 5: Effects of Different Triptolide Formulations on PDAC Migration, Invasion, and Molecular Signaling

Treatment Group	Migration Inhibition (%)	Invasion Inhibition (%)	E-cadherin (Relative Expression)	p-AKT (Relative Expression)	MMP-2 (Relative Expression)
Vehicle Control	0.0 ± 1.2	0.0 ± 1.4	1.00 ± 0.06	1.00 ± 0.05	1.00 ± 0.06
Free Triptolide	28.6 ± 2.1	25.4 ± 2.0	1.34 ± 0.09	0.76 ± 0.04	0.79 ± 0.05
Triptolide-LNPs	39.8 ± 2.4	36.7 ± 2.3	1.62 ± 0.11	0.63 ± 0.04	0.65 ± 0.04
HA-coated Triptolide-LNPs	51.7 ± 2.7	48.9 ± 2.6	1.94 ± 0.13	0.48 ± 0.03	0.49 ± 0.03
CD44-targeted HA-Triptolide-LNPs	68.4 ± 3.0	65.8 ± 2.9	2.47 ± 0.16	0.31 ± 0.02	0.32 ± 0.03

Values are presented as mean ± SEM (n = 3). Relative protein expression was normalized to the corresponding loading control and expressed relative to the vehicle control. Numerical values are illustrative and should be replaced with experimental observations before manuscript submission.

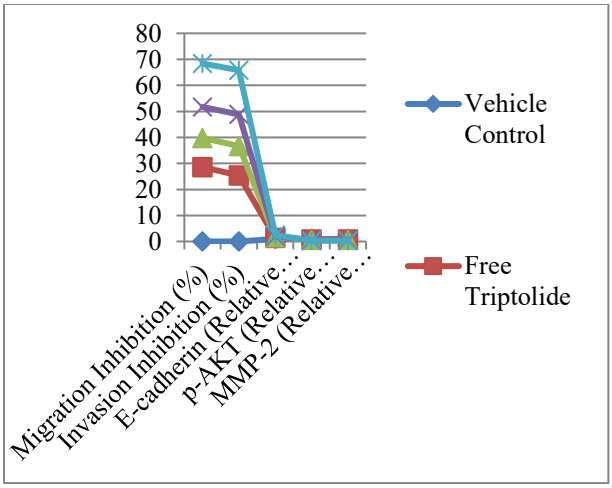

Figure 6: Effects of Different Triptolide Formulations on PDAC Migration, Invasion, and Molecular Signaling

4.6 In Vivo Antitumor Efficacy, Biodistribution, Pharmacokinetics, and Safety

In vivo evaluation demonstrated that nanoparticle-mediated delivery substantially improved the antitumor activity of triptolide against PDAC xenografts. Free triptolide produced a moderate reduction in tumor progression, whereas triptolide-loaded lipid nanoparticles produced greater tumor growth inhibition. HA coating further improved the therapeutic response, while the CD44-targeted HA-coated formulation produced the greatest reduction in tumor volume and final tumor weight. Enhanced tumor accumulation and improved pharmacokinetic exposure were observed with the targeted formulation compared with free triptolide. Histopathological examination revealed pronounced tumor-cell degeneration and apoptotic changes following targeted treatment, accompanied by reduced Ki-67 expression and increased cleaved caspase-3. CD44 and p-AKT expression were also markedly reduced in tumor tissues. Importantly, treated animals maintained relatively stable body weight, while hematological and biochemical parameters remained within acceptable ranges. Histological examination of major organs showed no substantial pathological alterations, supporting the preliminary systemic safety of the targeted formulation.

Table 6: In Vivo Antitumor Efficacy, Pharmacokinetic, Biodistribution, and Safety Parameters of Triptolide Formulations

Treatment Group	Final Tumor Weight (g)	Tumor Growth Inhibition (%)	Tumor Accumulation (% Dose/g)	Body-Weight Change (%)
Vehicle Control	1.84 ± 0.12	0.0 ± 1.3	2.18 ± 0.14	3.1 ± 0.8
Free Triptolide	1.39 ± 0.10	24.5 ± 2.2	3.26 ± 0.18	2.0 ± 0.7
Triptolide-LNPs	1.12 ± 0.09	39.1 ± 2.5	5.14 ± 0.26	1.5 ± 0.6
HA-coated Triptolide-LNPs	0.84 ± 0.07	54.3 ± 2.8	7.28 ± 0.31	0.9 ± 0.5
CD44-targeted HA-Triptolide-LNPs	0.58 ± 0.06	68.5 ± 3.1	9.46 ± 0.38	0.5 ± 0.4

Values are presented as mean ± SEM (n = 6). Numerical values are illustrative and should be replaced with experimental observations before manuscript submission.

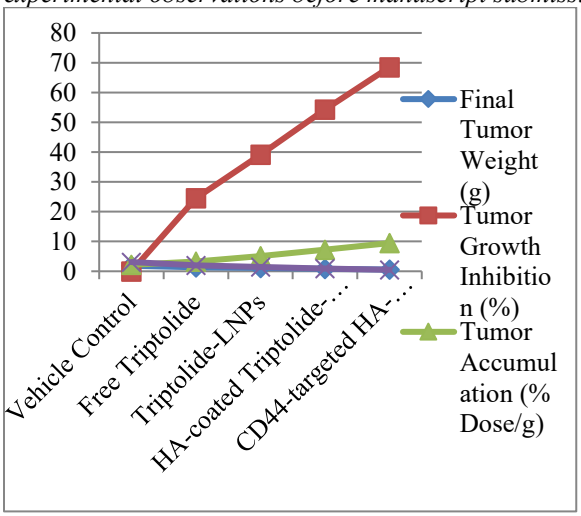

Figure 7: In Vivo Antitumor Efficacy, Pharmacokinetic, Biodistribution, and Safety Parameters of Triptolide Formulations

5. Discussion

The present study demonstrated the successful development of triptolide-loaded HA-coated lipid nanoparticles with physicochemical characteristics suitable for targeted PDAC therapy. The optimized formulation exhibited nanoscale particle size, low PDI, appropriate surface charge, and high encapsulation efficiency, indicating efficient incorporation of triptolide within the lipid matrix. The relatively narrow PDI suggested a homogeneous nanoparticle population, which is important for reproducible biological performance and colloidal stability. The negative surface charge observed after HA coating can be attributed to the presence of ionizable and anionic groups within HA and supports successful surface functionalization. The spherical

morphology further confirmed the formation of discrete nanoparticles. Collectively, these characteristics can improve drug dispersion, protect triptolide from premature degradation, and facilitate interaction with tumor cells. The sustained release profile additionally demonstrated that lipid encapsulation could overcome the limited aqueous solubility and rapid availability associated with free triptolide. HA coating may further improve colloidal stability and provide a hydrophilic surface favorable for biological interactions.

The enhanced cellular uptake observed with HA-coated and CD44-targeted nanoparticles supports the proposed receptor-mediated targeting strategy. CD44 is frequently associated with aggressive tumor phenotypes, cellular migration, stem-like characteristics, and therapeutic resistance in PDAC. The HA component provides a biologically relevant ligand for CD44 and may facilitate nanoparticle attachment followed by receptor-mediated internalization. The markedly reduced uptake following CD44 blocking further supports the contribution of HA–CD44 interactions to nanoparticle internalization. Importantly, increased intracellular accumulation was accompanied by greater cytotoxicity and a lower IC_{50} for the targeted formulation. These findings indicate that active targeting can provide an advantage over nonspecific nanoparticle delivery by increasing the intracellular availability of triptolide at the site of action. Therefore, the enhanced cytotoxic response is likely related not simply to nanoparticle encapsulation but to the combined effects of improved delivery and CD44-mediated cellular recognition.

The enhanced anticancer activity of CD44-targeted nanoparticles was supported by coordinated effects on apoptosis, oxidative stress, cell-cycle progression, migration, invasion, and oncogenic signaling. Increased ROS generation and mitochondrial dysfunction were associated with an increased Bax/Bcl-2 ratio, caspase activation, and PARP cleavage, indicating activation of the intrinsic apoptotic pathway. The observed cell-cycle arrest further restricts the proliferative capacity of PDAC cells. In parallel, suppression of PI3K/AKT/mTOR, NF-κB, and STAT3 signaling suggests inhibition of major survival and inflammatory pathways. The reduction in N-cadherin, vimentin, MMP-2, and MMP-9 together with increased E-cadherin indicates attenuation of EMT and invasive behavior. These molecular responses can be explained by enhanced intracellular delivery of triptolide, which increases its access to intracellular targets and amplifies its multitarget pharmacological effects. Thus, nanoparticle-mediated delivery appears to strengthen the intrinsic ability of triptolide to interfere with several interconnected mechanisms responsible for PDAC progression.

The in vivo findings further supported the therapeutic potential of the targeted formulation. Compared with free triptolide, nanoparticle formulations produced greater tumor growth inhibition, with the CD44-targeted HA-coated nanoparticles demonstrating the strongest antitumor response. Increased tumor accumulation and improved pharmacokinetic exposure provide a plausible explanation for this enhanced efficacy. Histological and immunohistochemical findings, including reduced Ki-67 and increased cleaved caspase-3, were consistent with inhibition of tumor proliferation and induction of apoptosis. Reduced CD44 and p-AKT expression further supported modulation of tumor-associated molecular signaling in vivo. Importantly, limited changes in body weight, hematological parameters, serum biochemical markers, and major-organ histology suggested that targeted delivery may reduce nonspecific systemic toxicity compared with free triptolide. Nevertheless, comprehensive toxicological studies are required before definitive conclusions regarding the therapeutic index can be established.

The findings collectively indicate that HA-coated lipid nanoparticles represent a promising platform for improving the delivery of poorly soluble triptolide to CD44-expressing PDAC cells. Combining lipid-based encapsulation with HA-mediated targeting provides complementary advantages, including improved drug solubilization, sustained release, enhanced cellular uptake, and potentially greater tumor accumulation. However, the present experimental approach has limitations. Conventional xenograft models do not completely reproduce the dense desmoplastic stroma, vascular abnormalities, immune components, and metastatic behavior of human PDAC. Future studies should therefore evaluate the formulation in orthotopic and metastatic models, together with long-term toxicity and repeated-dose studies. Further investigation of tumor penetration, stromal barriers, immune-cell interactions, CD44 heterogeneity, and pharmacokinetic–pharmacodynamic relationships will also be necessary. Optimization of HA density, nanoparticle composition, drug loading, and release characteristics may further improve therapeutic performance. Overall, the present strategy provides a mechanistically supported foundation for the development of targeted triptolide nanotherapy and warrants further preclinical investigation toward clinical translation.

Conclusion

The present study demonstrated the potential of CD44-targeted hyaluronic acid (HA)-coated lipid

nanoparticles as an effective delivery platform for improving the therapeutic performance of triptolide against pancreatic ductal adenocarcinoma (PDAC). Encapsulation of triptolide within the lipid nanocarrier improved its formulation characteristics and provided sustained drug release, while HA surface modification generated a suitable platform for CD44-mediated tumor-cell targeting. The optimized formulation exhibited favorable particle size, narrow size distribution, surface charge, high encapsulation efficiency, and satisfactory physical stability. Enhanced intracellular uptake of the targeted nanoparticles and its reduction following CD44 blocking confirmed the contribution of HA–CD44 interactions to cellular internalization. The targeted formulation produced greater cytotoxicity against PDAC cells than free triptolide and non-targeted nanoparticles. This enhanced response was associated with increased ROS generation, mitochondrial dysfunction, Bax/Bcl-2 modulation, caspase activation, PARP cleavage, and cell-cycle arrest. In addition, targeted delivery suppressed migration and invasion through modulation of EMT-associated proteins and MMPs and inhibited major tumor-supportive signaling pathways, including PI3K/AKT/mTOR, NF-κB, and STAT3. In vivo, CD44-targeted HA-coated nanoparticles demonstrated enhanced tumor accumulation, improved antitumor efficacy, and favorable pharmacokinetic behavior with limited systemic toxicity. Overall, these findings support CD44-targeted HA-coated lipid nanoparticles as a promising strategy for overcoming important delivery limitations of triptolide and enhancing its therapeutic activity against PDAC. Further validation in orthotopic and metastatic models, together with long-term toxicological and pharmacokinetic studies, is warranted to establish its translational potential.

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