

Synthetic Lethality through Dual ISG15 and HMGCR Targeting Disrupts Metabolic Homeostasis and Eliminates Pancreatic Cancer Stem Cells

Wouter De Smet^{1*}, Liesbeth Van Dam¹, Pieter Janssens²

¹Department of Pancreatic Cancer Stem Cell Biology, Faculty of Medicine, KU Leuven, Leuven, Belgium.

²Department of Synthetic Lethality and Metabolic Homeostasis, Faculty of Medicine, Ghent University, Ghent, Belgium.

*E-mail ✉ wouter.desmet@kuleuven.be

Abstract

Precision oncology has emerged as a groundbreaking framework in cancer treatment, leveraging detailed molecular characterization to develop tailored therapeutic strategies. Extending our earlier findings on ISG15's contribution to the development of pancreatic cancer stem cells (CSCs), the present study uncovered simultaneous overexpression of ISG15 and HMGCR in pancreatic ductal adenocarcinoma (PDAC). Combined inhibition of these two targets produced a synthetic lethal effect, resulting in efficient CSC depletion and substantial hindrance of tumor development. To harness this cooperative vulnerability, we constructed a hyaluronic acid-coated liposomal nanocarrier (LNP-Kd/Statin) that simultaneously delivers ISG15-specific siRNA and statins that block HMGCR activity. At the molecular level, ISG15 physically associates with HMGCR; its reduction prevents K48-linked ubiquitin-mediated proteasomal breakdown of HMGCR in an ISGylation-independent manner. This stabilization elevates cholesterol levels to promote cell viability while concurrently diminishing essential biosynthetic intermediates, thereby sensitizing the cells. Comprehensive sterolomics analysis validated the resulting metabolic shift, directly connecting the breakdown of cholesterol homeostasis to CSC eradication. Collectively, these insights illuminate a previously unrecognized ISG15-HMGCR signaling axis that drives PDAC progression and introduce a nanotechnology-based platform for undermining cancer stemness by exploiting metabolic liabilities. The approach strengthens the clinical applicability of synthetic lethality concepts in precision oncology, particularly for refractory solid tumors.

Keywords: ISG15, Statins, HMGCR, Synthetic lethal, Pancreatic ductal adenocarcinoma

Introduction

Pancreatic cancer (PC) persists as one of the most aggressive and fatal neoplasms, characterized by a five-year survival rate under 10% attributable to delayed detection, swift advancement, and marked therapeutic resistance [1-3]. Cancer stem cells (CSCs) constitute a resilient subpopulation that fuels tumor origination, dissemination, relapse, and chemoresistance, rendering

them a prime focus for novel interventions [4-6]. Synthetic lethality—an approach in which simultaneous disruption of two nonessential genes causes cell death while single-gene disruption remains viable—has emerged as an effective strategy for selectively ablating CSCs without harming healthy tissues [7, 8]. Nonetheless, the identification of exploitable synthetic lethal partnerships and the delineation of their functional mechanisms in PC remain critical knowledge gaps.

Interferon-stimulated gene 15 (ISG15) exhibits negligible basal expression in healthy tissues but rises dramatically upon exposure to viruses or interferon (IFN) stimuli. Comparable to ubiquitination, ISG15 attaches covalently to substrate proteins in a process termed ISGylation, which can be reversed by specialized de-ISGylating proteases. ISG15 also circulates as a free

Access this article online

<https://smerpub.com/>

Received: 12 February 2025; Accepted: 02 May 2025

Copyright CC BY-NC-SA 4.0

How to cite this article: De Smet W, Van Dam L, Janssens P. Synthetic Lethality through Dual ISG15 and HMGCR Targeting Disrupts Metabolic Homeostasis and Eliminates Pancreatic Cancer Stem Cells. Arch Int J Cancer Allied Sci. 2025;5(1):265-81. <https://doi.org/10.51847/rqgB7OASv3>

monomer both inside cells and in the extracellular space [9-12]. Despite its documented overexpression in PC and its supportive function in sustaining CSC traits [13, 14], the gene's influence on metabolic susceptibilities is only beginning to be explored.

Metabolic vulnerability involves the systematic rewiring of cellular bioenergetics, the selective blockade of indispensable metabolic cascades, and the therapeutic targeting of cancer-specific metabolic addictions [15, 16]. It further capitalizes on unique tumor metabolic profiles to internalize toxic substrate analogs, thereby generating fatal metabolic imbalances [17, 18]. This paradigm holds strong potential for anticancer strategies. Intriguingly, drug-sensitivity assays in our laboratory showed that PC cells lacking ISG15 become markedly more responsive to statins, which inhibit 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), the rate-limiting enzyme in the mevalonate-cholesterol pathway [19, 20]. While separate suppression of ISG15 or statin administration only modestly impaired CSC generation, their joint application achieved virtually complete CSC elimination and triggered widespread cell death. Tissue microarray profiling of clinical and murine PC samples further established that increased ISG15 and HMGCR protein abundance correlates with higher tumor grades, even though ISG15 knockdown left mRNA levels unchanged. This inconsistency points to prominent post-translational regulatory processes.

Advances in nanotechnology have transformed drug delivery for PC by improving site-specific accumulation and decreasing systemic adverse effects [21, 22]. For instance, nanoparticle-encapsulated irinotecan has already reached clinical application [23-25]. Leveraging these innovations, we formulated a hyaluronic acid (HA)-decorated liposomal nanosystem for concurrent delivery of statins and ISG15-targeting genetic constructs. This platform synergistically perturbs cholesterol homeostasis and CSC function, resulting in strong growth inhibition in both patient-derived organoids and in vivo xenograft models.

From a mechanistic standpoint, ISG15 depletion stabilized HMGCR protein by impeding its K48-linked ubiquitination and subsequent proteasomal degradation. The resulting cholesterol buildup initially bolsters CSC survival; however, sterolomics profiling disclosed a simultaneous shortage of upstream biosynthetic intermediates (such as desmosterol and squalene oxide), thereby inducing profound metabolic stress. Hence, coordinated targeting of ISG15 and HMGCR through the

engineered nanoplateform creates a metabolic trap: transient cholesterol elevation provides short-term support. At the same time, the abrupt failure of intermediate supply undermines CSC integrity and halts tumor expansion. In summary, these discoveries define a novel synthetic lethal relationship between ISG15 and HMGCR in PC and pioneer a nanotechnology-driven modality to dismantle CSC-dependent malignancy by reprogramming cellular metabolism (Graphical Abstract).

Materials and Methods

Materials

Glyceryl monostearate (GMS), soy phosphatidylcholine (SPC), cholesterol, DSPE-PEG-HA (hyaluronic acid-conjugated lipid), dimethyldioctadecyl ammonium bromide (DDAB), and 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) were sourced from Shanghai Yuanye Bio-Technology (Shanghai, China). The plasmid expressing ISG15-targeted RNAi (shRNA sequence: 5'-CATGTCGGTGTCAGAGCTGAA-3') was obtained through custom synthesis from Shanghai Genechem Co., Ltd. Atorvastatin calcium, Cycloheximide (CHX), Aloxistatin (E64d), MG132, and Pepstatin A (purity > 98%) were supplied by Selleck.cn (Shanghai, China). Lentiviral vectors encoding ISG15-WT and the ISG15-MUT (G156/157A) variant were produced by Shanghai Genechem Co., Ltd.

Preparation of LNP-Kd/Statin

Cationic liposomes co-loaded with ISG15-RNAi plasmid and atorvastatin (denoted LNP-Kd/Statin) were assembled through a thin-film hydration followed by sonication. In short, the lipid components—GMS (10 mg), SPC (20 mg), cholesterol (10 mg), DDAB (10 mg), DSPE-PEG-HA (1 mg), and atorvastatin (1 mg)—were fully dissolved in 10 mL of a chloroform/ethanol mixture (5:1, v/v). The solvent was subsequently removed under vacuum using a Rotavapor R-300 (Büchi, Switzerland) to form a uniform thin lipid layer. Hydration of this film was performed with 10 mL nuclease-free ddH₂O accompanied by gentle agitation. The resulting dispersion was sonicated (40 kHz, 300 W) for 10 min at 45 °C, then passed through a 0.45- μ m polycarbonate filter to yield blank LNP-Statin particles. Plasmid encapsulation involved the slow addition of 0.65 mg of the ISG15-RNAi plasmid (prepared in 10 mM Tris-

EDTA buffer, pH 7.4) to the LNP-Statin suspension under continuous magnetic stirring (500 rpm) for 30 min. Matched control formulations (LNP and LNP-Kd) were manufactured using identical procedures.

Fluorescent labeling

To enable visualization in cellular internalization assays, Dil (1 mg) was incorporated into the lipid film along with atorvastatin, yielding fluorescently labeled Dil@LNP-Kd/Statin nanoparticles.

Physicochemical characterization

Size and Zeta Potential: Measurements of hydrodynamic size, polydispersity index (PDI), and surface charge (zeta potential) were performed in triplicate via dynamic light scattering on a Zetasizer Nano ZS90 instrument (Malvern Instruments, UK).

Morphology: Nanoparticle morphology was examined by transmission electron microscopy (TEM, JEM-2100plus, JEOL, Japan) at an accelerating voltage of 100 kV following negative staining with 2% phosphotungstic acid.

Drug Loading and Encapsulation Efficiency: After ultracentrifugation (13,000 ×g, 40 min), the quantity of unbound atorvastatin remaining in the supernatant was assessed using UV-vis spectrophotometry at 290 nm. Encapsulation efficiency (EE) and drug loading (DL) were determined according to the following equations:

$$\begin{aligned} \text{EE (\%)} &= (\text{Drug}_{\text{feed}} - \text{Drug}_{\text{supernatant}}) / \text{Drug}_{\text{feed}} \times 100 \\ \text{DL (\%)} &= (\text{Drug}_{\text{feed}} - \text{Drug}_{\text{supernatant}}) / \text{Total nanoparticle weight} \times 100 \end{aligned} \quad (1)$$

Results:

$$\begin{aligned} \text{EE} \sim \text{atorvastatin} &\sim 58.07 \pm 6.71\%; \\ \text{DL} \sim \text{atorvastatin} &\sim 1.12 \pm 0.10\%; \\ \text{EE} \sim \text{plasmid} &\sim \approx 100\%; \\ \text{DL} \sim \text{plasmid} &\sim 1.25\% \end{aligned} \quad (2)$$

Quantitative calibration

A standard curve demonstrating excellent linearity ($R^2 = 0.998$) was generated by plotting UV absorbance at 290 nm against atorvastatin concentrations ranging from 7.81 to 500.00 µM/mL.

Cell culture and maintenance

The human pancreatic ductal adenocarcinoma (PDAC) cell lines BxPC3 (ATCC® CRL-1687™) and SW1990 (GeneChem, Shanghai) were propagated in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Thermo Fisher Scientific) containing 10% heat-inactivated fetal bovine serum (FBS; Sigma-Aldrich), 100 IU/mL penicillin, and 100 µg/mL streptomycin (Sigma-Aldrich). Cells were cultured in a humidified incubator set at 37 °C with 5% CO₂, and the culture medium was refreshed every 48–72 h. All lines were periodically screened for mycoplasma using the MycoAlert™ kit (Lonza) and verified for identity through short tandem repeat (STR) analysis before experimental use.

Cytotoxicity assay

Cell survival was evaluated through the CCK-8 method. In summary, BxPC3 and SW1990 cells were plated at 4×10^3 cells per well in 100 µL of full growth medium within 96-well plates. After allowing 12 h for cell attachment, cultures were exposed to a range of LNP-Kd/Statin concentrations (0.1–100 µg/mL) or equivalent control nanoparticles. Following a 48 h exposure period, 10 µL of CCK-8 reagent (Dojindo Laboratories) was added per well, and incubation continued for an additional 2 h at 37 °C with 5% CO₂. Absorbance readings were taken at 450 nm on a microplate reader (BioTek Synergy H1), applying background correction at 650 nm. The percentage of viable cells was determined according to the equation:

$$\begin{aligned} \text{Viability} \\ (\%) &= (\text{A}_{\text{treatment}} - \text{A}_{\text{blank}}) / (\text{A}_{\text{control}} - \text{A}_{\text{blank}}) \times 100 \end{aligned} \quad (3)$$

Results are expressed as mean ± SD from three independent experiments, each performed with n = 6 replicates per condition.

In vitro cellular uptake analysis

Intracellular transport efficiency of the LNP-Kd/Statin formulation was investigated by confocal laser scanning microscopy (CLSM; Leica TCS SP8, Wetzlar, Germany). BxPC3 cells were seeded at 5×10^4 cells per well onto glass coverslips in 12-well plates and grown to approximately 60% confluency. Subsequently, cells were treated with Dil-labeled LNP-Kd/Statin (at a Dil concentration equivalent to 1 µg/mL) in serum-free DMEM for 8 h under standard culture conditions (37 °C,

5% CO₂). After treatment, samples were washed three times with ice-cold PBS, fixed with 4% paraformaldehyde for 15 min, and nuclei were stained with DAPI (1 µg/mL) for 5 min. Images were obtained with a 63× oil-immersion objective, using excitation/emission wavelengths of 549/565 nm for Dil and 358/461 nm for DAPI. Reconstructed Z-stack images (0.5 µm intervals) were generated using Leica LAS X software to examine the subcellular localization patterns of the nanoparticles.

Sterolomics profiling and quantitation

LipidALL Technologies carried out comprehensive sterol profiling in accordance with standardized procedures [26]. Briefly, lipids were isolated from pancreatic cancer tissue samples via an adapted Bligh-Dyer extraction protocol incorporating the following steps: (1) homogenization of tissue in a chloroform/methanol/water solvent system (2:2:1.8, v/v/v) supplemented with 0.01% butylated hydroxytoluene (BHT) to minimize oxidative damage; (2) induction of biphasic separation by addition of 1 M KCl to achieve a final chloroform/methanol/water ratio of 1:1:0.9. The organic lipid phase then underwent alkaline saponification in 500 µL of 1 N ethanolic potassium hydroxide (KOH) containing 5 µg butylated hydroxytoluene (BHT) at 37 °C for 1 h with orbital shaking at 1200 rpm. Following saponification, 50 µL of a deuterated internal standard solution was added to each extract, containing: d₆-Lanosterol (10 ng), d₅-Zymosterol (10 ng), d₇-Lathosterol (10 ng), d₇-7-Dehydrocholesterol (10 ng), d₇-Sitosterol (10 ng), and d₆-Cholesterol (50 ng; Avanti Polar Lipids, Alabaster, AL, USA).

LC-MS/MS analysis

The derivatized sterols were separated by UPLC using a Shimadzu 40 × 3B UPLC system fitted with a Kinetex C18 column (2.1 × 100 mm, 1.7 µm particle size; Phenomenex). The mobile phase consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile/isopropanol (1:1, v/v). Gradient elution was performed as follows: 0–2 min at 60% B (isocratic), 2–15 min with a linear gradient to 95% B, and 15–18 min for column re-equilibration at 60% B. Mass spectrometric analysis utilized a Sciex QTRAP 6500+ instrument in positive multiple reaction monitoring (MRM) mode. Quantitative measurements were obtained by isotope dilution using scheduled MRM (sMRM) transitions, with data processing performed in Analyst

1.7.1. Individual sterol calibration curves showed strong linearity ($R^2 > 0.99$) based on peak area ratios of the analyte to its corresponding internal standard [26].

CRISPR/Cas9-mediated ISG15 knockout in pancreatic cancer cells

ISG15 gene disruption was achieved in pancreatic cancer cells through a dual single-guide RNA (sgRNA) CRISPR/Cas9 approach. The sgRNAs (sgRNA-1: 5'-GTGGTGGACAAATGCGACGAA-3'; sgRNA-2: 5'-GCACCGTGTTCATGAATCTGC-3') were selected to target regions surrounding exon 2 of ISG15, promoting efficient frameshift mutations. A repair donor plasmid carrying 1.5 kb homology arms on each side was designed to facilitate replacement of the targeted genomic segment with a puromycin resistance cassette (PuroR) and an enhanced green fluorescent protein (EGFP) reporter via homologous recombination (HR). Simultaneous delivery of the dual sgRNA/Cas9 expression plasmid and the donor construct into BxPC3 and SW1990 cells was accomplished by lentiviral transduction at an MOI of 10. Parallel control cells were transduced with a non-targeting empty sgRNA vector. Puromycin selection (2 µg/mL) was initiated 72 h post-transduction and maintained for 7 days. Knockout efficiency was rigorously confirmed by Genomic PCR (targeted amplification of the modified locus), qRT-PCR (showing >95% decrease in ISG15 transcript levels in KO clones), and Western Blot analysis (complete absence of ISG15 protein detected with a specific antibody). EGFP-positive cell populations were isolated by fluorescence-activated cell sorting (FACS Aria III, BD Biosciences) and propagated for subsequent biological evaluations.

Colony formation assay

For assessment of long-term proliferative capacity, 200 cells per well were seeded into six-well plates (Corning, Acton, MA, USA) and incubated for 2 weeks under standard growth conditions. Resulting colonies were fixed with 4% paraformaldehyde for 15 min and visualized by staining with 0.1% crystal violet. Colonies containing more than 50 cells were manually quantified using light microscopy.

Sphere formation assay

Cells harvested during the exponential growth phase were collected and suspended in serum-free DMEM/F12 medium enriched with 20 mg/ml EGF (Sigma-Aldrich,

Saint Louis, MO, USA), 5 µg/ml Insulin (Sigma-Aldrich), and 2% B27 (Invitrogen, Carlsbad, CA, USA). This suspension was distributed into six-well Corning Spheroid Microplates. The culture medium was replenished every 3 days. Spheroid development was monitored by capturing images with an inverted microscope and manually counting the formed spheroids.

Nude mouse xenograft experiments

Subcutaneous injection of 1×10^7 BxPC3 cells was performed in BALB/c-nu/nu mice. Ten days after cell inoculation, animals harboring tumors were randomly assigned to four experimental arms: liposome/control, liposome/knockdown ISG15, liposome/statin drug, and liposome/knockdown ISG15 statin. Treatments were given once daily for 2–3 weeks. Throughout the study period, daily records were maintained regarding the animals' overall health parameters, including mental alertness, food and water consumption, external appearance, and body temperature. Administration volumes were calibrated according to current body weight measurements, while body weight changes and survival outcomes were systematically documented. All procedures involving animals received prior approval from the Institutional Animal Care Committee of Nanjing University of Chinese Medicine and adhered to its guidelines (202403A057).

Western blot analysis

Harvested cells were rinsed twice using ice-cold PBS and lysed in ice-cold RIPA buffer (Beyotime). Protein content in the cleared supernatants was measured with Bradford reagent (Bio-Rad) to enable equal loading. Normalized samples underwent electrophoresis on 4–12% Bis-Tris NuPage gels at 200 V for 60 min. Resolved proteins were electrotransferred onto nitrocellulose membranes, which were subsequently blocked for 1 h at room temperature with 3% BSA prepared in TBST buffer (50 mM Tris [pH 8.0, 4 °C], 150 mM NaCl, 0.1% Tween-20). Overnight incubation with primary antibodies at 4 °C followed. All primary antibodies were sourced from Cell Signaling Technology (CST). Membranes were then washed three times in TBST before incubation with HRP-conjugated secondary antibodies or streptavidin-HRP. After three further TBST washes, chemiluminescent signals were developed using ECL reagents.

RT-qPCR

Cellular RNA was isolated with the RNeasy® Mini Kit (QIAGEN) following the supplier's protocol. Reverse transcription was performed using Promega reagents, followed by quantitative real-time PCR to determine HMGCRC transcript abundance. All primer pairs were custom-designed and produced by Sangon Biotech.

Flow cytometry

Quantification of cancer stem cell subpopulations relied on flow cytometric analysis. Logarithmically growing BxPC3 and SW1990 cells (both parental control and ISG15 knockdown (KD) lines) were treated with either DMSO or statins for 24 h. Treated cells were collected and enzymatically dissociated into single-cell suspensions with 0.25% EDTA-free trypsin. Following two PBS washes ($300 \times g$, 5 min each), cell density was adjusted to 1×10^6 cells per 100 µL in PBS containing 1% BSA. Appropriate fluorochrome-conjugated antibodies along with matching isotype controls (CD133-APC and CD44-APC, Elabscience) were added as specified by the manufacturer, and labeling occurred in the dark at 4 °C for 30 min. Stained cells were washed once more with PBS and resuspended in 400 µL PBS for measurement. Acquisition was performed on a BD FACSCanto II instrument, and data analysis was performed using FlowJo software (version 10.0).

Statistics

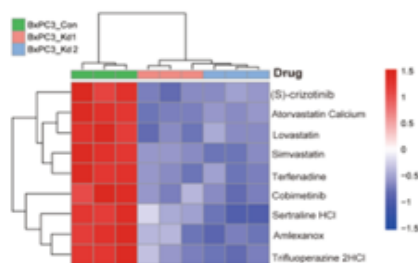
All statistical information, including significance thresholds, is presented within the figures and their respective legends. Group comparisons were conducted by one-way ANOVA with Dunnett's post hoc test for multiple comparisons. Each experiment was independently replicated three times, and quantitative results are reported as mean $\pm$ SD. Levels of statistical significance are marked as follows: * $p < 0.05$, ** $p < 0.01$, ns: not significant. Graphical representations were prepared in Prism10, and the mechanistic diagram was generated using BioRender (Agreement number: PG28T9IOLMA).

Results and Discussion

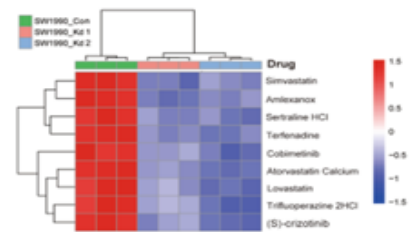
Loss of ISG15 sensitizes pancreatic cancer to statins via synthetic lethality

High-throughput screening of an FDA-approved compound collection demonstrated that depletion of ISG15 substantially heightened the vulnerability of pancreatic cancer (PC) cells to statin treatment (**Figures**

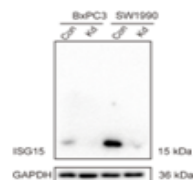
1a-1b). This finding was corroborated by establishing stable ISG15-knockdown lines in both BxPC3 and SW1990 cells via lentiviral transduction (**Figure 1c**). Subsequent dose-response studies using the CCK-8 assay examined the impact of varying statin concentrations in combination with ISG15 silencing. The data revealed robust inhibition of cellular proliferation when ISG15 knockdown was paired with statin exposure. Complementary flow cytometric profiling of CSC-associated markers CD44 and CD133 further confirmed a more substantial diminution of stem-like cell populations under the combined regimen. Clonogenic assays indicated that either ISG15 reduction or statin monotherapy achieved only limited suppression of long-term cell survival, whereas simultaneous application resulted in pronounced synergistic effects (**Figures 1d-1f**). Remarkably, although individual targeting modestly curtailed the self-renewal potential of cancer stem cells (CSCs) as evaluated by sphere formation, the dual intervention virtually eliminated the ability to generate spheroids (**Figures 1g-1i**). These observations underscore a clear synthetic lethal relationship and identify the ISG15-statin interaction as an innovative therapeutic avenue for overcoming stem cell-driven resistance mechanisms in PC.



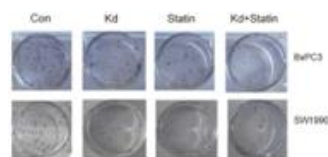
a)



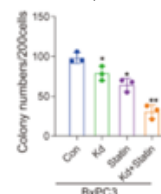
b)



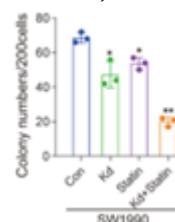
c)



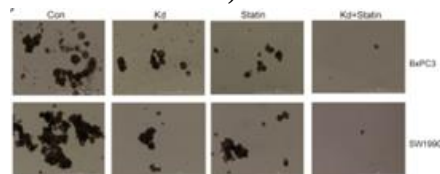
d)



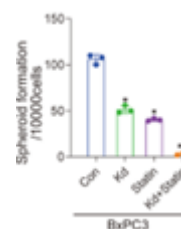
e)



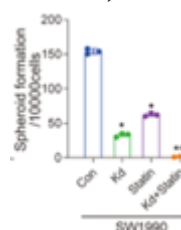
f)



g)



h)



i)

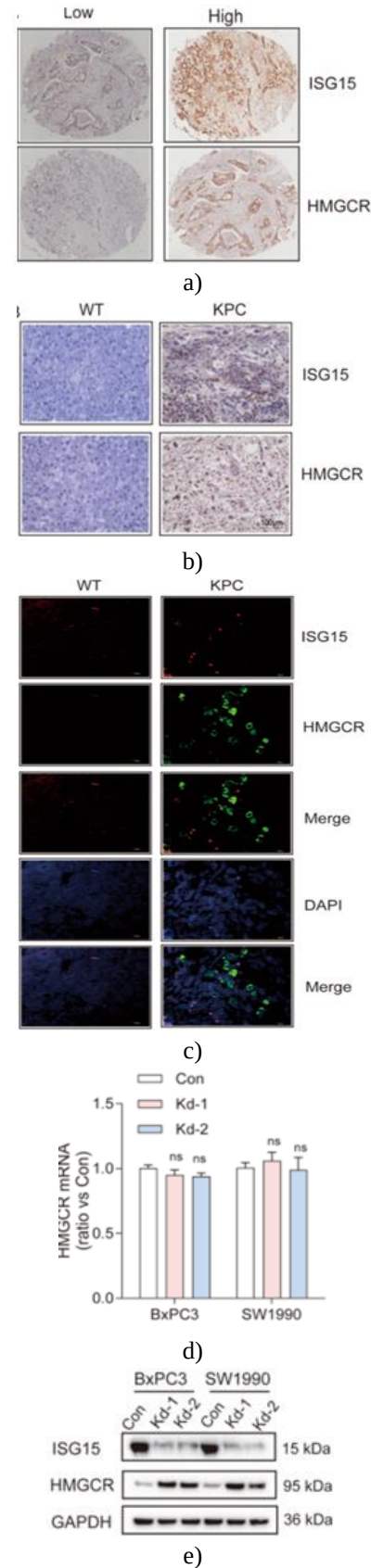
Figure 1. Combined ISG15 Knockdown and Statin Treatment Induces Synthetic Lethality in Pancreatic Cancer Cells

(a, b) Assessment of drug responsiveness in BxPC3 (a) and SW1990 (b) pancreatic cancer cells following exposure to test compounds in the setting of ISG15 depletion (ISG15 KD) compared with control (Con) cells

established through CRISPR/Cas9 editing, (c) Immunoblot analysis verifying the successful reduction of ISG15 protein levels in ISG15 KD cells compared with control cells, (d) Overview of the colony formation experimental design, where 200 individual cells per well received statin administration (20 μ M), ISG15 knockdown, or the dual intervention, (e, f) Numerical quantification of colonies formed by BxPC3 (e) and SW1990 (f) cells ($n = 3$ independent replicates per group, unpaired two-tailed Student's t test), (g) Representative micrographs depicting spheroid development in serum-free medium after a 10-day incubation period, (h, i) Counts of spheroids generated from BxPC3 (h) and SW1990 (i) cells ($n = 3$ per group, unpaired two-tailed Student's t test). All data are shown as mean $\pm$ SD. * $P < 0.05$; ** $P < 0.01$; ns, not significant.

ISG15 knockdown upregulates HMGCRCR protein expression and confers metabolic vulnerability in pancreatic cancer

To explore the clinical significance of the synthetic lethal relationship between ISG15 and HMGCRCR, their expression patterns were examined in pancreatic cancer (PC) specimens. Tissue microarray evaluation showed robust expression of both proteins in pancreatic tumors; however, they displayed a mutually exclusive distribution, whereby cells exhibiting high ISG15 levels possessed low HMGCRCR, and cells with elevated HMGCRCR displayed minimal ISG15 (**Figure 2a**). Compared with wild-type (WT) controls, both ISG15 and HMGCRCR protein levels were markedly elevated in the KRAS/p53-driven genetically engineered mouse model of pancreatic cancer (KPC) (**Figure 2b**). Furthermore, these proteins exhibited pronounced spatial segregation, occupying distinct niches within the tumor microenvironment, consistent with the heterogeneity observed in human disease (**Figure 2c**). Interestingly, ISG15 depletion (Kd) left HMGCRCR mRNA expression unchanged (**Figure 2d**) yet substantially elevated HMGCRCR protein levels (**Figures 2e-2f**), indicative of regulatory control occurring at the post-transcriptional level.



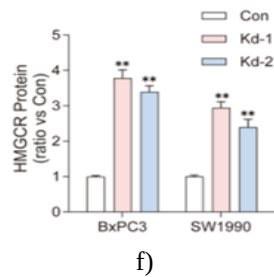


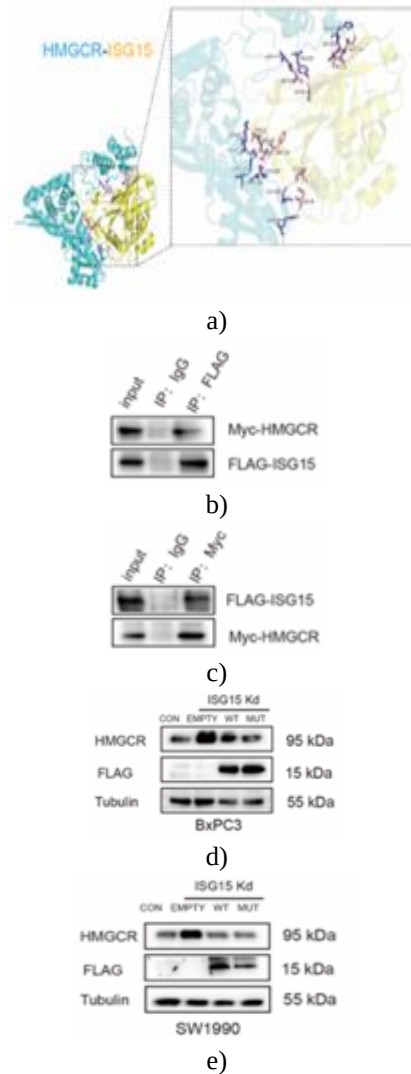
Figure 2. ISG15 Knockdown Stabilizes HMGR Protein Expression in Pancreatic Cancer

(a) Immunohistochemical (IHC) examination of ISG15 and HMGR protein distribution in human pancreatic ductal adenocarcinoma (PDAC) tissue microarrays, revealing that elevated expression of either protein is associated with more advanced tumor stages, (b, c) Immunohistochemical (b) and immunofluorescence (c) analyses of ISG15 and HMGR in Kras/p53-driven murine PDAC (KPC) models, demonstrating overall upregulation alongside spatially distinct localization patterns within malignant lesions, (d) Quantitative RT-PCR measurement of HMGR transcript abundance in BxPC3 and SW1990 cells following ISG15 knockdown (KD) relative to control (Con) cells ($n = 3$ per group, one-way ANOVA with Tukey's post hoc test), (e, f) Representative Western blot images (e) and densitometric quantification (f) of HMGR protein abundance in ISG15 KD cells, indicating post-translational stabilization ($n = 3$ per group, one-way ANOVA with Tukey's post hoc test). All experiments were independently repeated at least three times. Results are expressed as mean $\pm$ SD. *, $P < 0.05$; **, $P < 0.01$; ns, not significant.

ISG15 deficiency stabilizes HMGR by preventing ubiquitin-proteasome-mediated degradation in pancreatic cancer, independent of ISGylation

To investigate potential direct binding between ISG15 and HMGR, molecular docking was first applied to predict their interaction, followed by co-immunoprecipitation (CO-IP) experiments to validate the physical association (**Figures 3a-3c**). To examine whether ISG15 regulates HMGR in an ISGylation-dependent manner, ISG15 mutants lacking ISGylation activity (G156/157A) were constructed. These mutants failed to alter HMGR expression, confirming that the observed regulation is independent of ISGylation (**Figures 3d and 3e**). To define the mechanism driving HMGR accumulation upon ISG15 depletion, protein

stability was examined under proteostatic stress. Cycloheximide (CHX) chase experiments demonstrated that removal of ISG15 substantially increased the half-life of HMGR (**Figures 3f-3h**), reflecting greater protein stability. By comparison, treatment with the proteasome inhibitor MG132 eliminated differences in HMGR abundance between control and ISG15-deficient cells (**Figures 3i-3k**), revealing that ISG15 loss selectively disrupts HMGR turnover through the ubiquitin-proteasome system. Further mechanistic experiments established that ISG15 knockdown suppresses K48-linked ubiquitination of HMGR, thereby blocking its proteasomal degradation, whereas K63-linked ubiquitination remains unaltered (**Figures 3l-3n**).



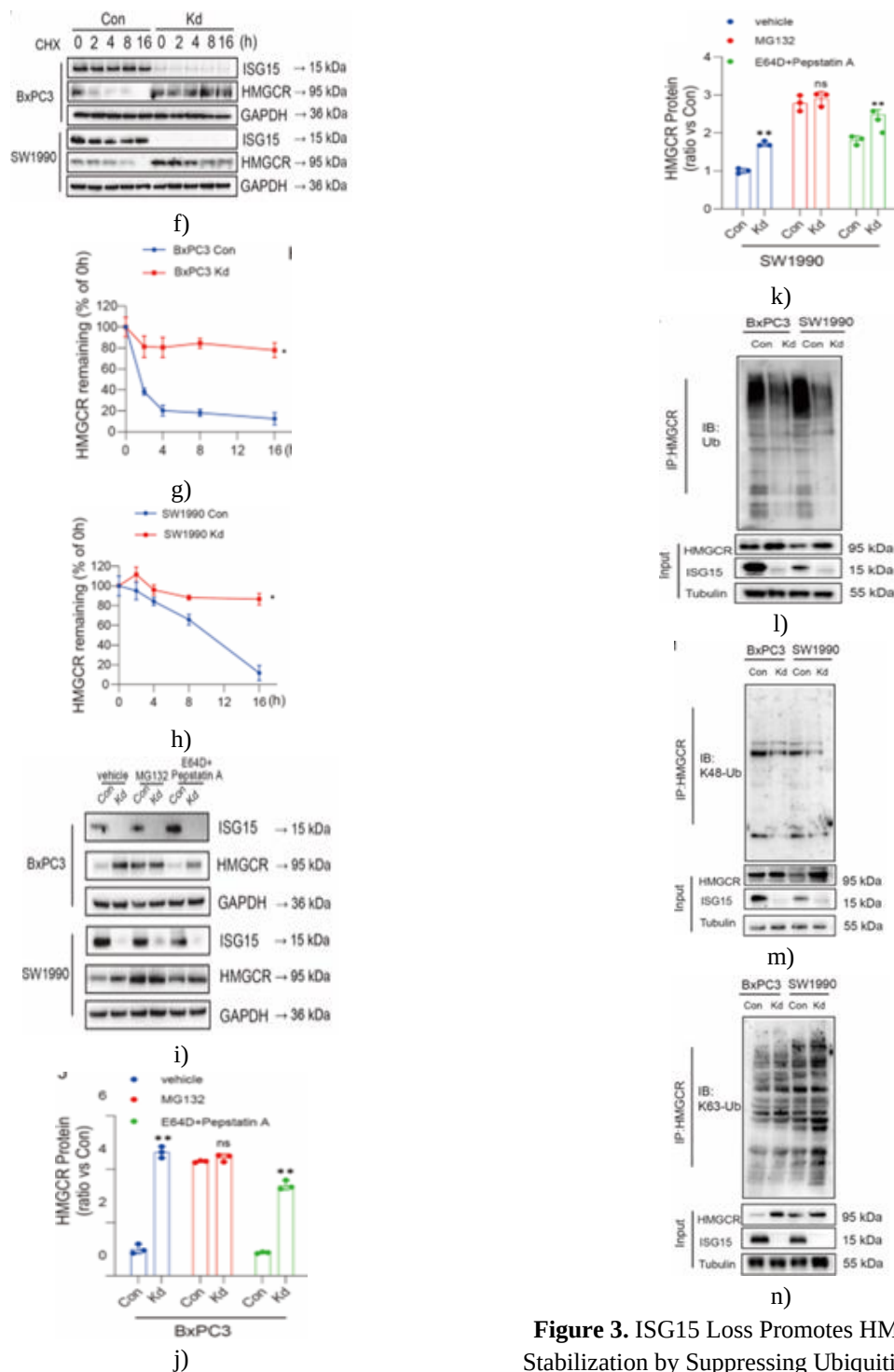


Figure 3. ISG15 Loss Promotes HMGR Protein Stabilization by Suppressing Ubiquitin-Proteasomal Degradation, Independently of ISGylation

(a) Molecular docking analysis predicting the interaction interface between ISG15 and HMGR, (b, c) Validation of the direct binding between ISG15 and HMGR proteins through co-immunoprecipitation (CO-IP) experiments, (d, e) Western blot analysis of HMGR

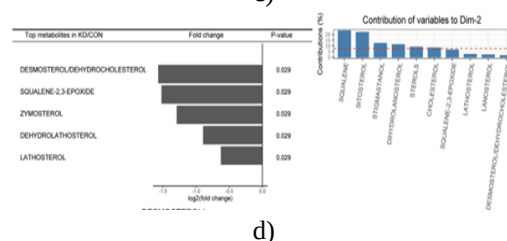
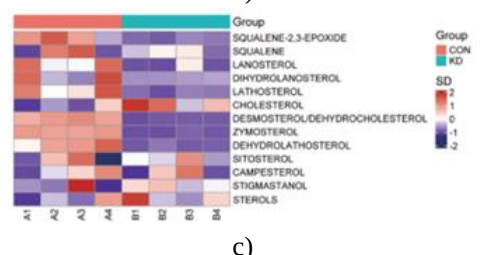
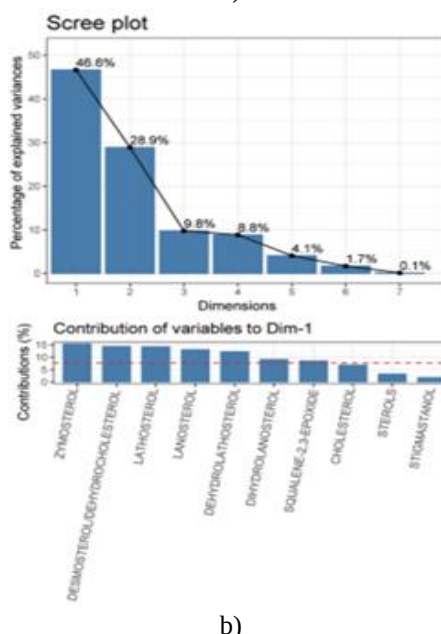
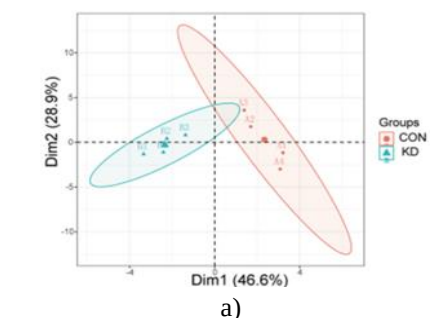
expression in ISG15-knockdown BxPC3 and SW1990 cells following transfection with plasmids encoding either wild-type ISG15 or the ISGylation-incompetent mutant (G156/157A) for 24 h, (f) Representative Western blots depicting HMGCR protein decay in control (Con) versus ISG15-knockdown (KD) BxPC3 and SW1990 cells after cycloheximide (CHX) treatment to halt de novo protein synthesis, (g, h) Calculated half-life ($t_{1/2}$) of HMGCR in BxPC3 (g) and SW1990 (h) cells, revealing significantly prolonged stability in the absence of ISG15 ($n=3$ per group, two-way ANOVA with Sidak's multiple comparisons test), (i) Immunoblotting of HMGCR levels in ISG15 KD cells exposed to the proteasome inhibitor MG132 or the autophagy-lysosome inhibitors E64D/Pepstatin A, (j, k) Densitometric quantification of HMGCR protein in BxPC3 (j) and SW1990 (k) cells following proteostatic blockade. Treatment with MG132 abrogated the elevation of HMGCR observed in ISG15-deficient cells, establishing dependence on proteasomal degradation ($n=3$ per group, unpaired two-tailed Student's t-test). (l-n) Assessment of overall HMGCR ubiquitination (l), K48-linked ubiquitin chains (m), and K63-linked ubiquitin chains (n) by Western blot in ISG15-knockdown BxPC3 and SW1990 cells. Experiments were independently repeated at least three times. Data represent mean $\pm$ SD. Statistical analyses were performed using unpaired Student's t-test or one-way ANOVA followed by Tukey's test. *, $P < 0.05$; **, $P < 0.01$; ns, not significant.

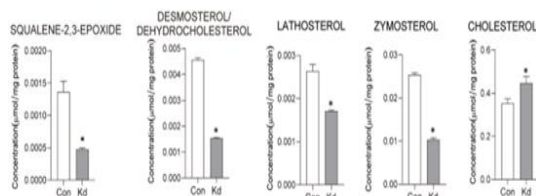
Collectively, these observations position ISG15 as an important controller of HMGCR protein fate. Its depletion disrupts ubiquitin-mediated proteasomal targeting, leading to HMGCR accumulation. Such stabilization sustains cholesterol production while lowering critical metabolic precursors, thereby opening an opportunity for selective cell killing through statin-triggered synthetic lethality.

ISG15 silencing leads to cholesterol buildup and interrupts biosynthetic flow in the cholesterol pathway within pancreatic cancer cells

To explore the downstream metabolic effects of reduced ISG15 expression, a comprehensive sterolomics analysis was performed in pancreatic cancer (PC) cell lines. Principal component analysis (PCA) showed distinct clustering of control and ISG15-knockdown samples across the initial two components (Dim-1: 46.6%; Dim-2: 28.9%), which together captured 76% of the overall

variance (**Figure 4a**). The application of 95% confidence ellipses verified clear group separation free of anomalous data points. Corresponding loading plots highlighted the dominant contribution of the ten most influential lipids to these dimensions, which largely comprised cholesterol synthesis intermediates (**Figure 4b**).





e)

Figure 4. ISG15 Reduction Alters Cholesterol Metabolic Networks and Exposes a Select Vulnerability in Pancreatic Cancer Cells

(a) Principal component analysis (PCA) applied to sterolomics datasets obtained from control (Con, Group A) and ISG15-knockdown (KD, Group B) pancreatic cancer cells. The 95% confidence ellipse highlights distinct metabolic profiles (Dim-1: 46.6%; Dim-2: 28.9%; cumulative variance: 76%) (n = 4 per group), (b) Loading plot identifying the ten lipids exerting the greatest influence on PCA separation, with cholesterol pathway intermediates being most prominent, (c) Heatmap displaying relative abundances of cholesterol-associated metabolites, characterized by widespread lipid reduction yet increased cholesterol (red) in ISG15 KD cells (hypothesis test, SQUALENE-2,3-EPOXYDE, $P = 0.036551699$; LANOSTEROL, $P = 0.042861789$; LATHOSTEROL, $P = 0.024469078$; CHOLESTEROL, $P = 0.034967532$; DESMOSTEROL/DEHYDROCHOLESTEROL, $P = 0.0000495$; ZYMOSTEROL, $P = 0.0000000146$; DEHYDROLATHOSTEROL, $P = 0.008821069$), (d) Volcano plot illustrating metabolites with significant differential abundance ($|\log_2FC| > 1$, $P < 0.05$) (n = 4 per group, Mann-Whitney U test), and (e) Box plots summarizing the four most substantially decreased cholesterol precursors (desmosterol/dehydrocholesterol, squalene-2,3-epoxide, zymosterol, lathosterol) along with cholesterol itself (n = 4 per group, unpaired Student's t test). Samples underwent analysis in three technical replicates. Data are mean $\pm$ SD. *, $P < 0.05$; **, $P < 0.01$; ns, not significant.

Heatmap inspection further highlighted that ISG15 knockdown produced a general decline in lipid metabolites across the board, contrasted by an unanticipated increase in cholesterol concentration (Figure 4c). Detailed measurements of pathway intermediates confirmed statistically significant reductions in critical precursors including desmosterol/dehydrocholesterol, squalene-2,3-epoxide, zymosterol, dehydro-lathosterol, and lathosterol

(Figures 4d and 4e). Collectively, these observations suggest that ISG15 loss impedes cholesterol production immediately after the HMGCR-mediated reaction, resulting in diminished precursor pools concurrent with cholesterol accumulation.

This shift in metabolic balance establishes a specific liability: stabilized HMGCR continues to support essential cholesterol needs for membrane maintenance, yet the pronounced shortage of upstream intermediates severely constrains pathway flexibility. Consequently, cells become critically reliant on ongoing HMGCR function, providing the mechanistic basis for the observed synthetic lethality when combined with statin therapy.

Synthesis and characterization of HA-Coated LNP-Kd/Statin nanoplatform

ISG15 depletion is suggested to stabilize the HMGCR protein primarily by hindering its ubiquitin-proteasome-mediated breakdown. This stabilization augments cholesterol production, thereby supporting the survival of pancreatic cancer cells. However, the adaptive response also exhausts essential upstream intermediates in the cholesterol pathway (such as squalene and zymosterol). Consequently, the cells develop an increased dependence on continued HMGCR function. Pharmacological suppression of HMGCR with statins therefore synergizes with ISG15 silencing, amplifying metabolic distress and facilitating tumor cell death. This exploitable weakness has already been documented through in vitro synergistic lethality studies. To extend these observations to an in vivo context, a specialized nanoparticle delivery vehicle was engineered for further validation.

The hyaluronic acid (HA)-functionalized liposomal nanoparticle designated LNP-Kd/Statin was fabricated employing the thin-film hydration method coupled with sonication (Figure 5a). Statins were initially incorporated into HA-modified liposomes to create LNP-Statin particles, which were then electrostatically assembled with plasmids encoding ISG15-targeting siRNA, resulting in the complete dual-loaded LNP-Kd/Statin system. Transmission electron microscopy (TEM) analysis verified the formation of well-defined spherical nanostructures exhibiting an average core diameter of 120.7 ± 9.0 nm (Figure 5b). Measurements by dynamic light scattering (DLS) yielded a hydrodynamic diameter of 197.7 ± 9.3 nm and a zeta potential of $+33.23 \pm 0.96$ mV, compared with

$+43.83 \pm 1.01$ mV for the statin-only LNP-Statin formulation (**Figure 5c**). This attenuation in positive surface charge was due to the binding of negatively charged siRNA plasmids that partially counterbalanced the cationic lipid DDAB.

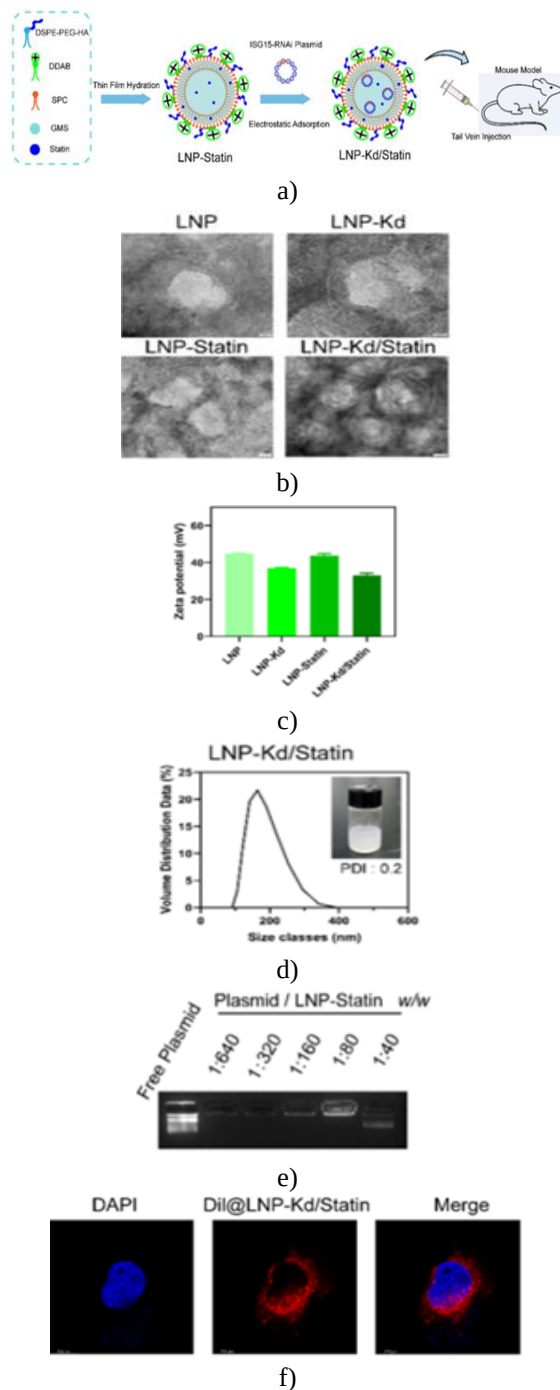


Figure 5. Construction and Detailed Characterization of HA-Coated LNP-Kd/Statin Nanoparticles

(a) Schematic overview of the assembly process for LNP-Kd/Statin: HA-modified liposomes pre-loaded with statins (LNP-Statin) were complexed with ISG15-specific siRNA plasmids through electrostatic interactions to produce the dual-payload nanocarrier, (b) Representative transmission electron microscopy (TEM) images illustrating uniform spherical morphology along with a measured core diameter of 120.7 ± 9.0 nm. Dynamic light scattering (DLS) determined the hydrodynamic diameter to be 197.7 ± 9.3 nm, including the hydration shell, (c) Surface charge evaluation by zeta potential: LNP-Kd/Statin displayed $+33.23 \pm 0.96$ mV, a reduction relative to the $+43.83 \pm 1.01$ mV of LNP-Statin resulting from siRNA neutralization effects, (d) Encapsulation efficiency (EE) and drug loading (DL) parameters for statins (EE: ; DL:) and siRNA (EE: $\sim 100\%$; DL: 1.25%), (e) Time-dependent release profile of siRNA from the nanopatform under simulated physiological conditions over 72 h, and (f) Confocal microscopic visualization of intracellular uptake of Dil-labeled LNP-Kd/Statin (red fluorescence) in BxPC3 cells, with nuclei stained blue by DAPI (scale bar: 20 μ m). Each sample was assessed in 3 technical replicates. Data are mean $\pm$ SD, and statistical comparisons were performed using unpaired Student's t-test. *, $P < 0.05$; **, $P < 0.01$; ns, not significant.

Both the statin drug and the siRNA component achieved excellent encapsulation and loading capacities (**Figures 5d and 5e**). Cellular internalization efficiency was examined by treating BxPC3 cells with fluorescently labeled nanoparticles, and confocal microscopy confirmed robust uptake and intracellular distribution (**Figure 5f**). Taken together, these data establish the reliable fabrication of a biocompatible, dual-loaded nanopatform with optimal physicochemical properties for potential targeted therapeutic use.

LNP-Kd/Statin nanotherapy inhibits growth of pancreatic cancer organoids and xenografts by concurrently targeting ISG15 and HMGCR

The therapeutic effectiveness of the LNP-Kd/Statin formulation was examined after initial confirmation of its biocompatibility. Control liposomes induced negligible cytotoxicity in BxPC3 cells, supporting the safety of the carrier system. Four distinct treatment conditions were then compared: (1) untreated control, (2) ISG15 gene silencing (Kd), (3) statin administered alone, and (4) the complete LNP-Kd/Statin dual-delivery system.

Treatment of BxPC3-derived organoids demonstrated that single interventions (ISG15 knockdown or statin exposure) achieved only partial growth restraint. In contrast, the combined LNP-Kd/Statin approach led to almost total cessation of organoid expansion (**Figure 6a**). Histological examination using hematoxylin-eosin (HE) staining at day 7 highlighted severe disorganization in the dual-treatment specimens, including breakdown of epithelial structures and widespread nuclear condensation (**Figure 6b**). Immunofluorescence assays further established that the nanotherapy effectively reduced both ISG15 protein abundance and HMGCR levels within the organoids (**Figure 6c**), verifying successful engagement of the intended molecular targets.

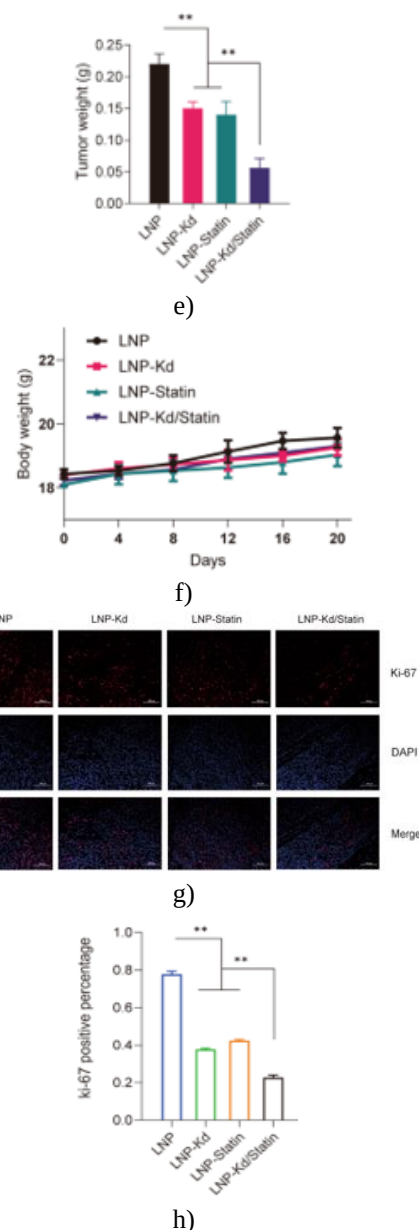
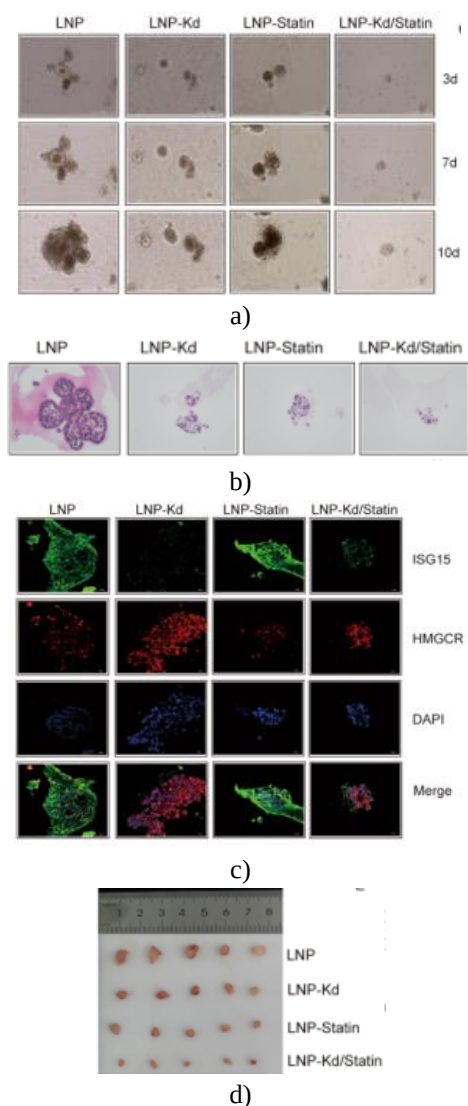


Figure 6. LNP-Kd/Statin Nanotherapeutic Approach Restrains Pancreatic Cancer Organoids and Xenograft Tumors through Integrated ISG15-HMGCR Inhibition:

(a) Temporal monitoring of organoid growth in BxPC3 models following exposure to control (Con), ISG15 knockdown (KD), statin alone (Statin), or LNP-Kd/Statin combination, evaluated on days 3, 7, and 10, (b) Hematoxylin and eosin (H&E) histology of organoids at day 10, depicting pronounced structural collapse in the combination treatment arm (scale bars: 100 μ m), (c) Immunofluorescent labeling showing decreased ISG15 (green) and HMGCR (red) signals in organoids receiving

LNP-Kd/Statin (nuclei counterstained with DAPI in blue; scale bars: 50 μ m), (d) Overview of the in vivo experimental design: Athymic nude mice bearing BxPC3 xenografts were administered nanotherapy via tail vein injection commencing on day 10 after tumor inoculation. Tumors were excised on day 28 (n = 5 per group), (e) Comparison of excised tumor masses among all treatment groups (n = 5 per group, one-way ANOVA with Tukey's multiple comparisons test), (f) Serial body weight recordings across the treatment period, revealing no evidence of systemic adverse effects (n = 5 per group, one-way ANOVA with Tukey's multiple comparisons test), (g) Immunofluorescence evaluation of Ki-67 expression in tumor sections from LNP-Kd/Statin-treated mice relative to controls (n = 5 per group, one-way ANOVA with Tukey's multiple comparisons test), and (h) Quantitative determination of the proportion of Ki-67-positive cells within xenograft tumors (n = 5 per group, one-way ANOVA with Tukey's multiple comparisons test). Data are mean $\pm$ SD. *, $P < 0.05$; **, $P < 0.01$; ns, not significant.

In subcutaneous BxPC3 xenograft models established in nude mice, systemic delivery of LNP-Kd/Statin produced marked antitumor responses, decreasing tumor volume by 65% ($P < 0.01$) and tumor weight by 58% ($P < 0.01$) compared with controls, with superior efficacy compared with individual monotherapies (**Figures 6d and 6e**). Throughout the study, the animals' body weights remained stable (**Figure 6f**), indicating limited overall toxicity. Molecular analysis of harvested tumors corroborated the dual suppression of ISG15 and HMGCR proteins, mirroring results obtained in culture. Proliferation marker assessment via Ki-67 staining demonstrated robust antiproliferative activity in the combination arm (**Figures 6g-6h**). Importantly, histopathological evaluation confirmed the absence of organ toxicity associated with the formulation.

In summary, the LNP-Kd/Statin nanoplatform induces effective synthetic lethality by simultaneously modulating ISG15 and HMGCR pathways. This dual-targeting strategy successfully eliminates both cancer stem-like cells and proliferating tumor bulk while exhibiting favorable systemic tolerability.

Synthetic lethality targeting cholesterol homeostasis through ISG15 and HMGCR

Pancreatic ductal adenocarcinoma (PDAC), the third most frequent cause of cancer mortality [27], continues to present formidable clinical challenges. Although

incremental progress has been achieved with chemotherapy and immunotherapy [28-32], the persistence of cancer stem cells (CSCs) and the metabolic flexibility of PDAC cells frequently undermine treatment success. The present study introduces an innovative dual-intervention framework that combines synthetic lethality principles with advanced nanotechnology. This approach disrupts CSC-mediated resistance and metabolic adaptation, offering a transformative approach to PDAC management.

Diverging from earlier investigations that emphasized ISG15 involvement in autophagy or immune modulation [14, 33, 34], the current work uncovers its previously unrecognized role in governing cholesterol metabolism. Concurrent suppression of ISG15 and pharmacological inhibition of HMGCR establish a synthetic lethal relationship. This interaction depletes critical biosynthetic intermediates (e.g., squalene oxide, zymosterol) while unexpectedly increasing end-product cholesterol levels. The resulting metabolic "double bind" restricts CSC adaptive capacity by limiting precursor availability, even as excess cholesterol disrupts membrane-associated signaling essential for survival. Such a strategy surpasses conventional single-pathway targeting by harnessing metabolic vulnerability to eliminate resilient CSC populations.

A precision nanoplatform enabling dual payload delivery and stromal penetration

Although liposomal formulations of irinotecan and nab-paclitaxel have established the clinical relevance of nanomedicine in PDAC [23-25], the HA-coated LNP-Kd/Statin system advances this field substantially. By simultaneously transporting ISG15-directed siRNA and statins, the platform facilitates selective tumor accumulation through CD44 receptor-mediated endocytosis [35-37], effectively navigating the formidable stromal barrier that impedes standard treatments [38, 39]. Its ability to achieve coordinated suppression of ISG15 via RNA interference and HMGCR via enzymatic blockade, all while minimizing systemic adverse effects, fulfills a critical therapeutic gap in pancreatic ductal adenocarcinoma (PDAC) management by providing multi-pathway precision without the toxicity burden associated with traditional combination regimens.

Immunometabolic interactions and intratumoral heterogeneity

Of particular interest is the observation that, despite elevated expression of both ISG15 and HMGR in pancreatic cancer, these proteins exhibit spatially distinct patterns, with ISG15-high and HMGR-high cell populations occupying separate tumor compartments in a mutually opposing fashion. Beyond metabolic alterations, ISG15 depletion was found to downregulate PD-L1 and promote CD8⁺ T-cell recruitment [33], indicating immune-potentiating effects not typically associated with statin monotherapy. This reciprocal distribution implies possible compensatory mechanisms or functional opposition between ISG15-driven immune control and HMGR-mediated metabolic adaptation during pancreatic tumor development. The combined immunometabolic influence of the LNP-Kd/Statin platform thus serves as a connector between molecularly targeted agents and immunotherapeutic modalities, with potential to counteract the highly immunosuppressive PDAC tumor microenvironment [28-32].

Pathways to clinical application: combination regimens and biomarker-guided approaches

The synthetic lethal relationship arising from ISG15-HMGR co-inhibition provides a rational foundation for developing mechanism-based therapeutic combinations. In contrast to empirical high-throughput screening methods, this strategy deliberately links a ubiquitin-proteasome regulator (ISG15) with a key metabolic enzyme inhibitor (statin) to enhance tumor selectivity. Future investigations should focus on three key directions to facilitate clinical advancement. First, biomarker-enriched clinical trials will be crucial for linking pretreatment ISG15 and HMGR levels to treatment outcomes, supporting personalized patient selection. Second, strategies aimed at stromal modification—such as co-administration of LNP-Kd/Statin with hyaluronidase to degrade hyaluronic acid-rich extracellular matrix—may improve nanoparticle distribution and efficacy in fibrotic tumors. Third, capitalizing on ISG15's capacity to suppress PD-L1 and enhance CD8⁺ T-cell infiltration could enable synergistic integration with checkpoint inhibitors, thereby sensitizing immunologically “cold” PDAC lesions. Collectively, these directions hold promise for overcoming the complex obstacles to effective PDAC therapy and translating synthetic lethality concepts into clinical practice.

Conclusion

In conclusion, integrating synthetic lethality concepts, nanotechnology, and detailed sterolomics profiling has enabled the development of a metabolism-directed therapeutic strategy that exploits a critical weakness in PDAC—its reliance on the flexibility of the cholesterol pathway. This research recharacterizes ISG15 as a central modulator of pancreatic cancer metabolism and provides a framework for future nanotherapeutic designs that can circumvent treatment resistance. As precision oncology increasingly emphasizes multi-pronged, microenvironment-responsive interventions, the current platform illustrates the powerful synergy between mechanistic insight and technological innovation in addressing refractory malignancies.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

References

1. Halbrook CJ, Lyssiotis CA, Pasca di Magliano M, Maitra A, Tuveson DA, Crawford HC, et al. Pancreatic cancer: advances and challenges. *Cell*. 2023;186(8):1729-54.
2. Stoffel EM, Brand RE, Goggins M. Pancreatic cancer: changing epidemiology and new approaches to risk assessment, early detection, and prevention. *Gastroenterology*. 2023;164(5):752-65.
3. Wang J, Yang J, Chen Y, Li X, Zhang L, Liu W, et al. Consensus, debate, and prospective on pancreatic cancer treatments. *J Hematol Oncol*. 2024;17(1):92.
4. Zhu K, Xie V, Huang S. Epigenetic regulation of cancer stem cell and tumorigenesis. *Adv Cancer Res*. 2020;148:1-26.
5. Loh JJ, Ma S. Hallmarks of cancer stemness. *Cell Stem Cell*. 2024;31(5):617-39.
6. Saw PE, Chen J, Song E, Liu X, Zhao Y, Xu J, et al. Cancer stem cell mimicry for immune evasion and therapeutic resistance. *Cell Stem Cell*. 2024;31(8):1101-12.
7. Ngoi NYL, Tan AC, Wong AL, Lim JS, Tan DS, Yap TA, et al. Synthetic lethal strategies for the development of cancer therapeutics. *Nat Rev Clin Oncol*. 2025;22(1):46-64.

8. Previtali V, Zaffaroni N, Daidone MG, Folini M, Giordano S, Riva F, et al. New horizons of synthetic lethality in cancer: current development and future perspectives. *J Med Chem.* 2024;67(14):11488-521.
9. Mathieu NA, Paparisto E, Barr SD, Binari R, Hagan C, Cordeiro A, et al. HERC5 and the isgylation pathway: critical modulators of the antiviral immune response. *Viruses.* 2021;13(6).
10. Mirzalieva O, Juncker M, Schwartzenburg J, Gallo A, Desai S, DeBruyne D, et al. ISG15 and isgylation in human diseases. *Cells.* 2022;11(3).
11. Qin Y, Wu Y, Liu H, Wang X, Sun Z, Zhang M, et al. Posttranslational isgylation of NLRP3 by HERC enzymes facilitates inflammasome activation in models of inflammation. *J Clin Invest.* 2023;133(20).
12. Qin Y, Wu Y, Liu H, Wang X, Sun Z, Zhang M, et al. ISGylation by HERCs facilitates STING activation. *Cell Rep.* 2024;43(5):114135.
13. Sun J, Zhang L, Zhao Y, Wang H, Chen X, Liu Q, et al. Loss of TRIM29 suppresses cancer stem cell-like characteristics of PDACs via accelerating ISG15 degradation. *Oncogene.* 2020;39(3):546-59.
14. Meng Y, Wu J, Li Z, Wang F, Chen Y, Liu T, et al. ISG15 promotes progression and gemcitabine resistance of pancreatic cancer cells through ATG7. *Int J Biol Sci.* 2024;20(4):1180-93.
15. Merteroglu M, Santoro MM. Exploiting the metabolic vulnerability of circulating tumour cells. *Trends Cancer.* 2024;10(6):541-56.
16. Finley LWS. What is cancer metabolism? *Cell.* 2023;186(8):1670-88.
17. Luengo A, Gui DY, Vander Heiden MG. Targeting metabolism for cancer therapy. *Cell Chem Biol.* 2017;24(9):1161-80.
18. Xiao Y, Xu X, Zhang P, Li H, Wang J, Chen L, et al. Emerging therapies in cancer metabolism. *Cell Metab.* 2023;35(8):1283-303.
19. Jiang R, Li Y, Zhang H, Wang S, Chen M, Liu X, et al. Statins in mitigating anticancer treatment-related cardiovascular disease. *Int J Mol Sci.* 2024;25(18).
20. Tripathi S, Gupta E, Galande S. Statins as anti-tumor agents: A paradigm for repurposed drugs. *Cancer Rep (Hoboken).* 2024;7(5):e2078.
21. Won EJ, Kim JH, Lee SH, Park JY, Choi YJ, Kim YS, et al. Gene therapy using nanocarriers for pancreatic ductal adenocarcinoma: applications and challenges in cancer therapeutics. *Pharmaceutics.* 2022;14(1).
22. Zhao X, Liu Y, Chen Z, Wang L, Li J, Yang H, et al. Penetration cascade of size switchable nanosystem in desmoplastic stroma for improved pancreatic cancer therapy. *ACS Nano.* 2021;15(9):14149-61.
23. Agarwal H, Sharma R, Singh A, Gupta P, Kumar S, Mehta K, et al. Theranostic nanoparticles for detection and treatment of pancreatic cancer. *Wiley Interdiscip Rev Nanomed Nanobiotechnol.* 2024;16(4):e1983.
24. Kumari S, Singh A, Kumar R, Sharma V, Gupta S, Thakur M, et al. Polysaccharides as therapeutic vehicles in pancreatic cancer. *Drug Discov Today.* 2025;30(3):104320.
25. Park W, Chawla A, O'Reilly EM. Pancreatic cancer: a review. *JAMA.* 2021;326(9):851-62.
26. Lam SM, Xu L, Li Y, Wang H, Tan B, Chen J, et al. A multi-omics investigation of the composition and function of extracellular vesicles along the temporal trajectory of COVID-19. *Nat Metab.* 2021;3(7):909-22.
27. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. *CA Cancer J Clin.* 2024;74(1):12-49.
28. Labori KJ, Lassen K, Dueland S, Grønbech JE, Sørbye H, Hoem D, et al. Neoadjuvant FOLFIRINOX versus upfront surgery for resectable pancreatic head cancer (NORPACT-1): a multicentre, randomised, phase 2 trial. *Lancet Gastroenterol Hepatol.* 2024;9(3):205-17.
29. Giordano G, Milella M, Salvatore L, Rossi L, Tortora G, Bria E, et al. Prognostic analysis and outcomes of metastatic pancreatic cancer patients receiving nab-paclitaxel plus gemcitabine as second or later-line treatment. *Cancer Med.* 2024;13(12):e7345.
30. Tempero MA, Malafa MP, Al-Hawary M, Behrman S, Benson AB, Binder L, et al. Pancreatic adenocarcinoma, version 2.2021, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw.* 2021;19(4):439-57.
31. Ye X, Zhang Y, Wang L, Chen H, Liu F, Zhao S, et al. Clinical immunotherapy in pancreatic cancer. *Cancer Immunol Immunother.* 2024;73(4):64.
32. Drougkas K, Karamouzis MV, Koutsoukos K, Kotsantis I, Karavasilis V, Gourgiotis S, et al. CAR-T cell therapy in pancreatic and biliary tract cancers: an updated review of clinical trials. *J Gastrointest Cancer.* 2024;55(3):990-1003.
33. Alcala S, Martinelli P, Sistigu A, Di Carlo E, Ciancaglini M, Proietti E, et al. ISG15 and isgylation

- is required for pancreatic cancer stem cell mitophagy and metabolic plasticity. *Nat Commun.* 2020;11(1):2682.
34. Burks J, Reed RE, Reddy S, Kolhe R, Fulzele S, Sahni S, et al. ISG15 pathway knockdown reverses pancreatic cancer cell transformation and decreases murine pancreatic tumor growth via downregulation of PDL-1 expression. *Cancer Immunol Immunother.* 2019;68(12):2029-39.
35. Bubin R, Uljanovs R, Strumfa I. Cancer stem cells in pancreatic ductal adenocarcinoma. *Int J Mol Sci.* 2023;24(8).
36. Biserova K, Jakovlevs A, Mikelsons A, Cekstere I, Briede I, Krumina Z, et al. Cancer stem cells: significance in origin, pathogenesis and treatment of glioblastoma. *Cells.* 2021;10(3).
37. Kang X, Zhang Y, Wang H, Chen L, Liu J, Sun Q, et al. Dual-cascade responsive nanoparticles enhance pancreatic cancer therapy by eliminating tumor-resident intracellular bacteria. *Adv Mater.* 2022;34(49):e2206765.
38. Large DE, Abdelmessih RG, Fink EA, Auguste DT. Liposome composition in drug delivery design, synthesis, characterization, and clinical application. *Adv Drug Deliv Rev.* 2021;176:113851.
39. Raza F, Zafar H, Khan S, Ahmad A, Ullah R, Khan M, et al. Liposome-based diagnostic and therapeutic applications for pancreatic cancer. *Acta Biomater.* 2023;157:1-23.