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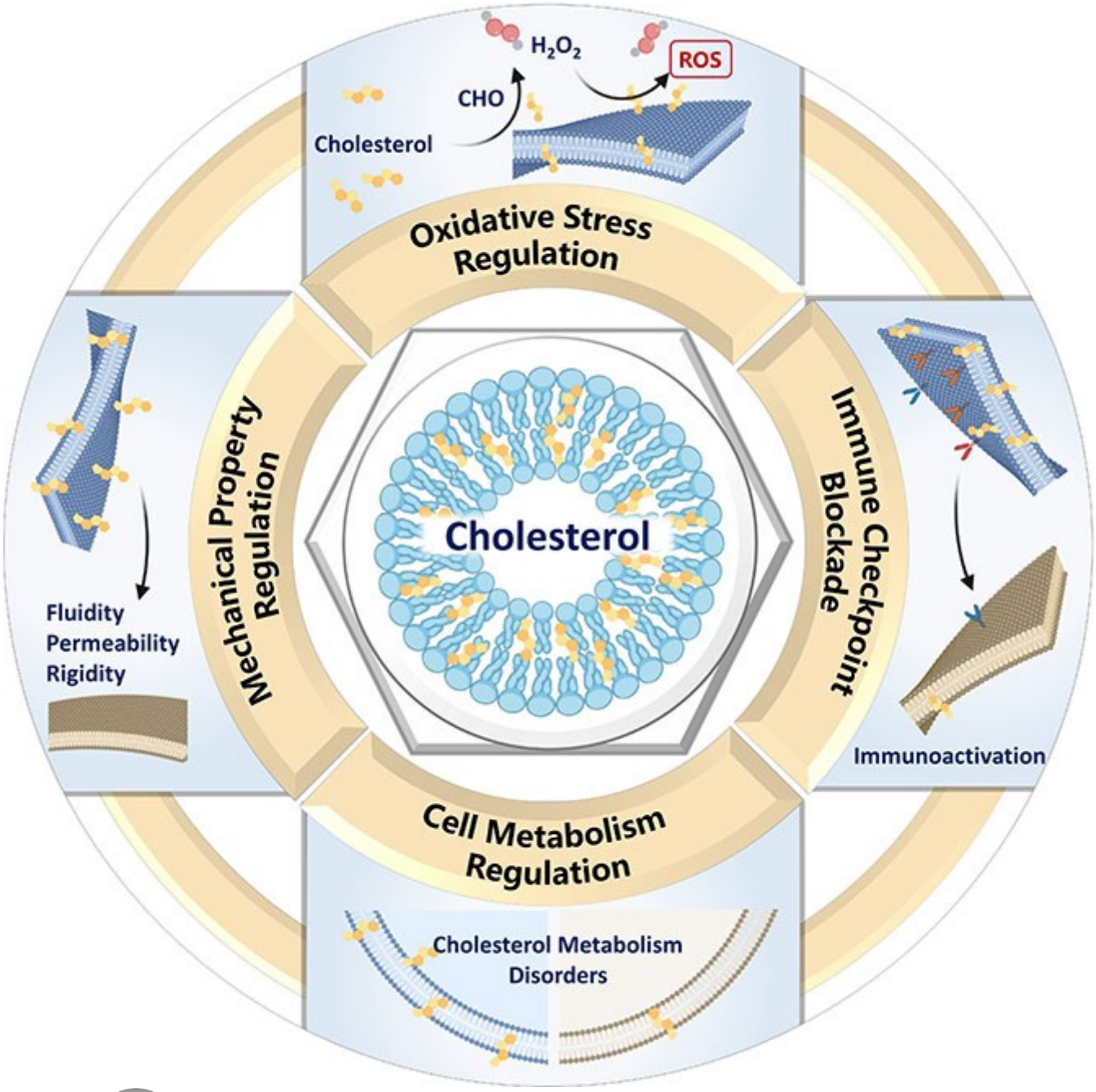
Tumor Therapy

Multifunctional Nanomaterials Mediate Cholesterol Depletion for Cancer Treatment

Wenyao Zhen[+], *Tomas Germanas*[+], *Ralph R. Weichselbaum, and Wenbin Lin**



Abstract: Cholesterol is an essential membrane component, and the metabolites from cholesterol play important biological functions to intricately support cancer progression and dampen immune responses. Preclinical and clinical studies have demonstrated the role of cholesterol metabolism regulation on inhibiting tumor growth, remodeling the immunosuppressive tumor microenvironment (TME), and enhancing anti-tumor immunity. In this minireview, we discuss complex cholesterol metabolism in tumors, its important role in cancer progression, and its influences on immune cells in the TME. We provide an overview of recent advances in cancer treatment through regulating cholesterol metabolism. We discuss the design of cholesterol-altering multifunctional nanomaterials to regulate oxidative stress, modulate immune checkpoints, manipulate mechanical stress responses, and alter cholesterol metabolic pathways. Additionally, we examine the interactions between cholesterol metabolism regulation and established cancer treatments with the aim of identifying efficient strategies to disrupt cholesterol metabolism and synergistic combination therapies for effective cancer treatment.

1. Introduction

As the most prevalent lipid species present in mammalian cells, cholesterol comprises up to 30% of total lipids in humans.[1] Its highly hydrophobic nature (log P = 8.7) and rigid structure often place it within lipid bilayers or store it in its esterified form within lipid droplets. Cholesterol plays an important role in establishing biochemical and biophysical functions of cell membranes, including organizing lipid microdomains, distributing receptors, and ensuring membrane integrity and fluidity.[2] The dysregulation of cholesterol metabolism stands as a hallmark of cancer progression,[3] influencing cell proliferation, migration, invasion and so on.[4] While normal mammalian cells synthesize cholesterol through the mevalonate (MVA) pathway,[2b] tumor cells accumulate high levels of cholesterol through the cholesterol-sequestering low-density lipoprotein receptor (LDLR) and increased cholesterol biosynthesis by acetyl coenzyme A (AcCoA) in the MVA pathway to support their proliferation. Numerous extrinsic factors also affect cholesterol metabolism in cancer cells. For instance, the hypoxic TME and the byproducts of glycolysis, including protons and CO_2 generated by cancer cells, can further stimulate cholesterol synthesis in the acidic tumor microenvironment (TME).[5]

In addition to cancer cells, the TME accommodates numerous immune-effector or immunosuppressive cells, including T lymphocytes, B lymphocytes, dendritic cells (DCs), tumor-associated macrophages, myeloid-derived suppressor cells (MDSCs), neutrophils, and natural killer cells.[6] Recent evidence indicates that high-cholesterol diet can lead to the accumulation of more immunosuppressive cells, such as polymorphonuclear neutrophils and T cells, and the depletion of cytotoxic $CD8^+$ T cells. Additionally, the accumulation of a high level of cholesterol in the TME has been shown to induce endoplasmic reticulum (ER) stress and T cell exhaustion, resulting in the upregulation of several immune checkpoints, including programmed cell death protein 1 (PD-1), T-cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), lymphocyte-activation gene 3 (LAG-3), and cluster of differentiation 244 (CD244, 2B4), thereby promoting tumor metastasis.[7]

Clinical research has indicated that cholesterol metabolism plays critical and diverse functions in the progression of colon, breast, and prostate cancers.[8] As a result, many approaches have been developed to deliver cholesterol-regulating drugs to tumor sites for tumor growth inhibition (TGI). To date, statins, a class of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) inhibitors that inhibit cholesterol biosynthesis by disrupting the MVA pathway, are the most explored drugs for cholesterol metabolism regulation.[9] Xu and co-workers recently revealed that unnatural peptide assemblies effectively reduce cholesterol levels in the cell membrane and, when combined with cholesterol-lowering agents, synergistically inhibit the proliferation of cancer cells in vitro.[10] However, the dense matrices and high heterogeneity of solid tumors limit the retention and effectiveness of these molecular drugs.[11] Nanoscale drug delivery systems (DDS) with optimal sizes, shapes, and surface charges can facilitate the simultaneous delivery of multiple drugs and their accumulation in tumors through the enhanced penetration and retention (EPR) effect.[12] Moreover, nanomaterials with optical, magnetic, catalytic, and other intrinsic properties can be integrated into the DDS to augment the therapeutic efficacy and overcome the acquired resistance to cancer therapy. Multifunctional nanoplatforms also have the capacity to reshape the immunosuppressive TME by alleviating tumor hypoxia, enhancing systemic antitumor immune responses, altering the metabolic pathways of cancer cells, and modifying the extracellular matrix (ECM). Thus, recent efforts have been devoted to constructing multifunctional nanoplatforms with cholesterol metabolism regulators to inhibit tumor growth and invasion.[13]

[*] Dr. W. Zhen,[+] T. Germanas,[+] Prof. Dr. W. Lin
Department of Chemistry
The University of Chicago
Chicago, Illinois 60637, United States
E-mail: wenbinlin@uchicago.edu

Dr. W. Zhen,[+] Prof. R. R. Weichselbaum, Prof. Dr. W. Lin
Department of Radiation and Cellular Oncology and Ludwig Center for Metastasis Research
The University of Chicago
Chicago, Illinois 60637, United States

[+] Dr. W. Zhen and T. Germanas contributed equally to this work.

This minireview provides an overview of cholesterol's pivotal roles in tumors, emphasizing its involvement in cancer progression and its influence on immune regulation. We further discuss recent therapeutic approaches targeting cholesterol regulation and explore their potential synergy with established anti-cancer treatments, including oxidative stress modulation, immune checkpoint downregulation, mechanical property regulation, and cell metabolism regulation. We offer insights into advancing cancer therapy through multi-functionalization of nanomaterials and the challenges in regulating cholesterol metabolism and cancer therapy. We anticipate this review will stimulate interest across various disciplines, prompting further investigations on cholesterol regulation and translation of effective and safe strategies into the clinic for cancer treatment.

2. Oxidative Stress Regulation

Reactive oxygen species (ROS) including superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and hydroxyl radical ($^\bullet$OH) participate in numerous physiological processes and serve as signaling molecules for cell proliferation. However, excessive ROS production causes oxidative stress to induce cell death. With remarkable advances in nanotechnology, a wide range of nanomaterials with ROS generation effects or ROS scavenging capacities have been designed, providing effective strategies to disrupt the antioxidant defenses of cancer cells and cause cancer cell death.

Cholesterol oxidase (CHO), a bifunctional flavoenzyme in the oxido-reductase family, catalyzes the oxidation of cholesterol by oxygen to the corresponding carbonyl product, cholest-4-ene-3-one (cholestenone), along with H_2O_2 as a metabolite.[11] The selective delivery of CHO to tumors can modulate cholesterol metabolism, inhibit tumor growth, and alter the tumor immune microenvironment to revitalize anti-tumor responses.[14] However, suitable carriers are needed to maintain the CHO activity in the TME.[15] As a class of crystalline materials composed of metal ion or cluster centers linked by organic ligands through coordination bonds,[16] nanoscale metal–organic frameworks (nMOFs) possess porous structures with high surface areas and represent a novel class of DDSs for drug delivery.[17] Du and co-workers prepared a CHO and doxorubicin (DOX) loaded Cu^{2+}-modified zirconium-based MOF (DOX@CHO-ZrMOF(Cu)) to oxidize cholesterol and produce H_2O_2 for the generation of highly cytotoxic $^\bullet$OH through Fenton-like reactions. The ROS pressure and chemotherapy synergistically enhanced tumor inhibition.[18] Zhao and co-workers recently synthesized a copper-dibenzo-[g,p]chrysene-2,3,6,7,10,11,14,15-octaol (Cu-DBCO/CL) nMOF with a rod-like morphology for the delivery of CHO and lysyl oxidase inhibitor (LOX-IN-3) to tumors (Figures 1a, 1b).[19] Cu-DBCO nMOF has a high CHO loading capacity of 52.9 wt %. In vivo, CHO catalyzes the conversion of oxygen and excess cholesterol into H_2O_2 and cholestenone to inhibit tumor growth. Furthermore, Cu-DBCO/CL catalyzes the conversion of O_2 and H_2O_2 into $O_2^{\bullet-}$ and $^\bullet$OH to disrupt mitochondrial function and induce cuproptosis. ROS-induced release of LOX-IN-3 modifies the ECM, promoting the maturation of DCs and facilitating the infiltration of $CD8^+$ T cells. The depletion of cholesterol by CHO further enhances the overall ROS level in the tumors via H_2O_2 production. The authors further showed that Cu-DBCO/CL disrupts cellular redox balance to exert cytotoxic effects on

Wenyao Zhen is a postdoctoral fellow under the joint supervision of Prof. Wenbin Lin and Prof. Ralph R. Weichselbaum at the University of Chicago. She obtained her Ph.D. degree from the University of Science and Technology of China (USTC) in December 2021. Her current research focuses on the design of stimuli-responsive, multifunctional nanoscale framework materials for cancer therapy.

Ralph R. Weichselbaum is the Daniel K. Ludwig Distinguished Service Professor of Radiation and Cellular Oncology Chair, Department of Radiation and Cellular Oncology. His research primarily focuses on radiation oncology and cancer immunotherapy.

Tomas Germanas is a pre-med undergraduate student in Chemistry and Biological Chemistry at the University of Chicago. He is currently conducting research in Dr. Wenbin Lin's lab, where he focuses on synthesizing novel nanoscale framework materials for cancer therapeutic applications.

Wenbin Lin is the James Franck Professor of Department of Chemistry and Department of Radiation and Cellular Oncology at the University of Chicago. His group has pioneered the studies of metal–organic frameworks in cancer therapy, bioimaging, earth-abundant metal catalysis, artificial photosynthesis, asymmetric catalysis, and second-order nonlinear optics. He is also the founder of two biopharmaceutical companies which have developed five clinical candidates based on the scientific discoveries made by his research group.

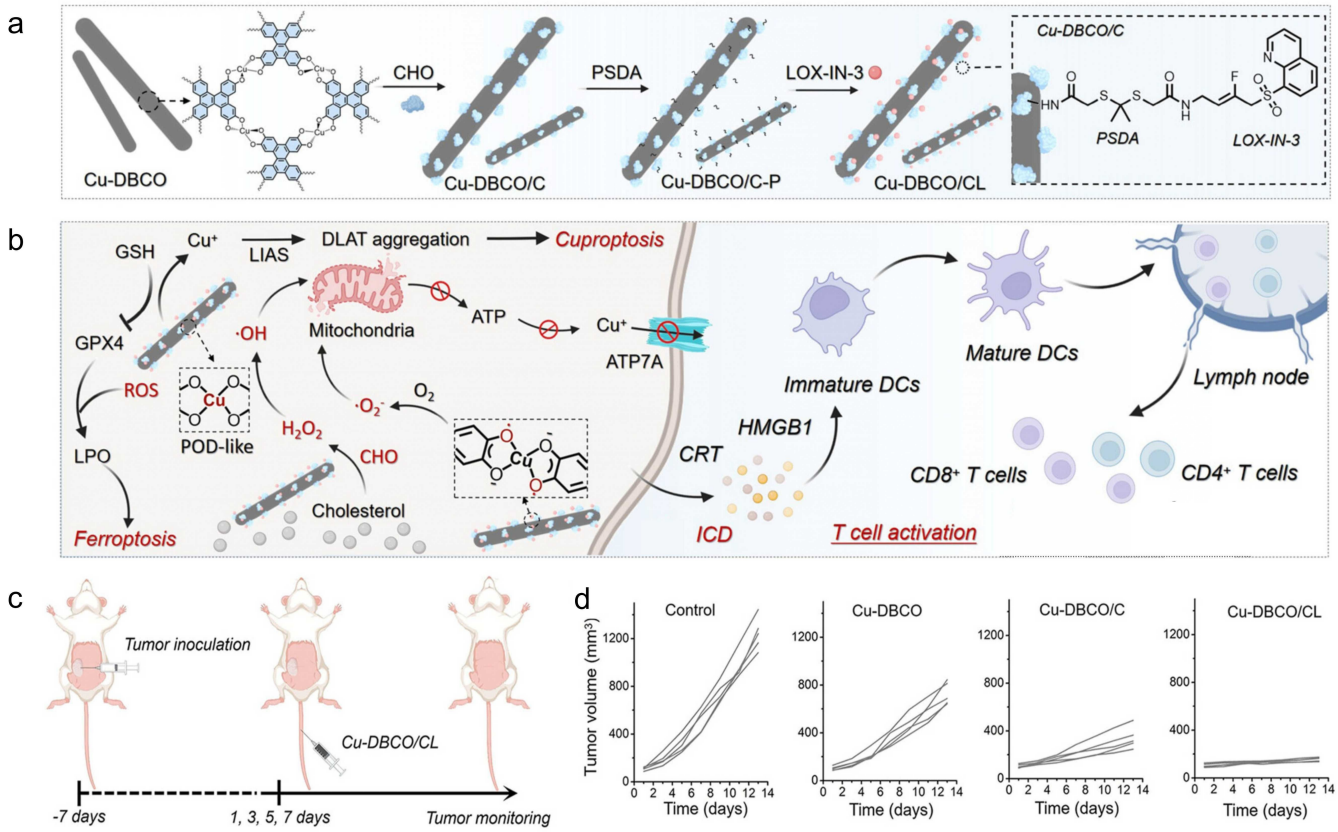


Figure 1. (a) Preparation of copper-dibenzo-[g,p]chrysene-2,3,6,7,10,11,14,15-octaol (Cu-DBCO/CL). (b) Schematic illustration of Cu-DBCO/CL-activated antitumor immune response. (c) Treatment schedule of orthotopic 4T1 tumor-bearing mice. (d) Tumor volumes after different treatments ($n = 5$).[19] Copyright 2024, American Chemical Society.

4T1 and MCF-7 cell lines and elicit strong antitumor effects on 4T1 tumor-bearing mouse model (Figures 1c, 1d). This work highlights the exciting potential of increasing intracellular ROS pressure by leveraging high levels of cholesterol in the tumors.

3. Immune Checkpoint Regulation

The presence of regulatory immuosuppressive cells, secretion of immunosuppressive cytokines and overexpression of multiple immune checkpoints compromise the efficiency of cancer immunotherapy. As cholesterol metabolism can alter membrane stiffness to impact the interactions between immune cells and tumor cells, several studies have been performed to uncover the relationships between cholesterol metabolism regulation and cancer immunotherapy.

3.1. Physical Immune Checkpoint Regulation through Modulating Cellular Biomechanics

Cellular rigidity is a biomechanical trait associated with cancer cell transformation, malignancy, and metastasis.[20] While tumors typically show increased stiffness compared to normal tissues, presumably due to abnormal production and crosslinking of ECM proteins,[21] individual cancer cells often exhibit softer mechanical properties compared to non-malignant cells. This softness has been attributed to increased levels of plasma membrane cholesterol.[22] Recent research indicates that mechanical abnormalities can significantly contribute to a diminished response to immune cells.[7] T cells play a dual role in the immune response: they not only sense mechanical cues in their environment but also apply forces at the immunological synapse to exert cytotoxicity against target cells.[23] When T cells interact with the surfaces of target cells, the mechanical attributes of the target cells' cortical structures, such as the plasma membrane and the underlying actin cortex, can impact their interactions.[24] Studies have shown that cytoskeletal forces and effector cytokine production are significantly reduced when T cells encounter a soft substrate surface or soft target cells.[23g,25] However, how cancer cell stiffness impacts an immune response remains poorly understood. It has been shown that cancer cells may exploit cellular softness as a form of physical immune checkpoint to resist T cell-mediated cytotoxicity by attenuating the mechanical forces exerted by T cells.[26]

Tang and co-workers demonstrated that the cortical structure of cancer cells is softened by a cholesterol-enriched plasma membrane. This alteration enables cancer cells to evade T-cell-mediated cytotoxicity, demonstrated by both in

vitro and in vivo experiments.[27] To counteract the evasion strategy of cancer cell, they targeted this mechanical immune checkpoint and attempted to modulate cancer cells stiffness by adjusting the plasma membrane cholesterol levels.[27] They employed methyl-β-cyclodextrin (Me-β-CD), a biocompatible compound commonly used as a solubilizer for hydrophobic drugs, to deplete cholesterol and consequently increase cellular stiffness.[28] Upon treatment with Me-β-CD, a notable enhancement in the stiffness of cancer cells was observed, which enhanced the antitumor efficacy through amplified T cell forces.

As T cells exhibit increased response against a stiffer surface upon T cell receptor (TCR) triggering, this work highlights the potential of mechanical checkpoint blockade as an innovative approach to enhance the effectiveness of T-cell-based immunotherapies by regulating cholesterol levels to manipulate cellular softness. In addition to stiffness, mechanical properties such as viscoelasticity may also facilitate tumor immune evasion.[29]

3.2. Immune Checkpoint Downregulation

Immune checkpoint blockade therapies have gained U.S. Food and Drug Administration (FDA) approval for treating various types of tumors by inhibiting checkpoints like PD-1 and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) to stimulate the immune response against tumors.[30] Like cancer cells, activated T cells undergo rapid proliferation and require sufficient cholesterol for expansion. Additionally, cholesterol plays an important role in establishing a mature immunological synapse. Sterol regulatory element-binding protein 2 (SREBP2) signaling is crucial for $CD8^+$ T cell proliferation and effector function. Conversely, oxysterols, which are enriched in the TME, may hinder T cell anti-tumor immunity through liver X receptor (LXR) activation. Cholesterol accumulation in the TME has been linked to inducing ER stress and exacerbating T cell exhaustion via upregulation of PD-1, TIM-3, 2B4, LAG-3, and other immune checkpoints. Therefore, it is crucial to assess cholesterol metabolism across various tumor-infiltrating T cell subsets, including effector versus memory, functional versus dysfunctional, and helper versus killer T cells. Furthermore, extrinsic and intrinsic cholesterol may play different roles. While enhancing intrinsic cholesterol biosynthesis and uptake may be advantageous, an imbalance in extrinsic cholesterol levels may lead to dysfunction of T cells.[31]

Zhang and co-workers developed a novel approach to regulate cholesterol metabolism for synergistic chemiexcited-photodynamic therapy (PDT) and non-antibody-dependent immunotherapy of glioblastoma multiforme (GBM) in mice. They synthesized a pegylated tetrakis(4-carboxyphenyl) porphyrin (TCPP)-based nanoscale hydrogen-bonded organic framework (nHOF) with the loaded chemiluminescent reagent, 2′,6′-dimethylcarbonylphenyl-10-sulfopropyl acridinium-9-carboxylate 4′-NHS ester (NDN). The resulting nanohybrid nHOF-NDN with a uniform size of 70 nm was coated with a $CaCO_3$ layer, and further surface-modified with CHO and LXR-623, a liver X receptor agonist. Finally, the nano-hybrid was surface functionalized with rabies virus glycoprotein 29 (RVG29), to afford HN@CaCL–R with the ability to cross the blood–brain barrier and accumulate at the glioma site (Figures 2a, 2b).[31d] Significant release of Ca^{2+} was observed at the lysosomal pH (≈5.0) and the TME pH (≈6.5). In contrast, only a small amount of Ca^{2+} was detected at the simulated normal tissue pH (≈7.4). CHO loaded in HN@CaCL–R enzymatically oxidizes cholesterol to produce H_2O_2. H_2O_2 can then stimulate chemiexcited PDT, leading to a robust release of damage-associated molecular patterns (DAMPs). In the TME, chemiexcited-PDT increased $CD8^+$ T cell infiltration via triggering immunogenic cell death (ICD) and overcame the penetration depth constraint of conventional PDT. In an acidic environment, $CaCO_3$ can degrade and promote the polarization of macrophages from the M2 to the M1 phenotype to reverse the suppressive immune microenvironment. Manipulation of the cholesterol content in the TME via the HN@CaCL–R nano-hybrid reduced the expression of PD-1 and 2B4 immune checkpoints (Figures 2c, 2d). HN@CaCL–R induced T-cell-mediated antitumor immunotherapy to suppress the growth of orthotopic GBM (Figures 2e–2h).

GBM exhibits abnormally high levels of cholesterol in the TME, posing a significant challenge for immunotherapy due to its highly immunosuppressive and resistant nature. Furthermore, glioma-supportive macrophages (GSMs) act as "cholesterol factories" with hyperactive cholesterol metabolism and efflux. These GSMs provide cholesterol to fuel GBM growth and induce exhaustion of $CD8^+$ T cells, exacerbating immunosuppressive TME.[32] The hyperactive cholesterol metabolism and efflux behavior of GSMs is distinct from that of GBM cells, providing a potential target for intervention. The enzyme 7-dehydrocholesterol reductase (DHCR7) converts 7-dehydrocholesterol (7-DHC) to cholesterol in cells,[33] serving as a bottleneck for cholesterol synthesis. As a result, silencing DHCR7 can inhibit cholesterol efflux from GSMs to reduce cholesterol levels in the TME and halt tumor progression without central nervous system toxicity.

Zhang, Zhao, Chen, Jiang, and co-workers utilized a "tumor organoid-on-chip" microfluidic platform to identify a DNA vector encoding DHCR7-silencing short hairpin RNA (shDHCR7).[34] They synthesized an intracavitary sprayable nanoregulator (NR)-encased hydrogel system to target the DHCR7 gene for regulating cholesterol metabolism in GSMs (Figure 3a).[34a] Glutathione (GSH)-triggered and lipid modified poly-β-amino-ester (PBAE) 447, C10-S-S-PBAE447 (CSP), was synthesized to deliver plasmids. Ionizable CSP was chemically altered with hydrophobic alkyl side chains to facilitate the formation of terpolymers under physiological conditions. This modification led to enhanced endosomal disruption and transfection efficiency. Mannose moieties were integrated into 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-poly (ethylene glycol) (DSPE-PEG) to augment GSM uptake. Nuclear targeting was accomplished by functionalizing PBAE447 with nuclear localization signals (NLS). The researchers then used micro-

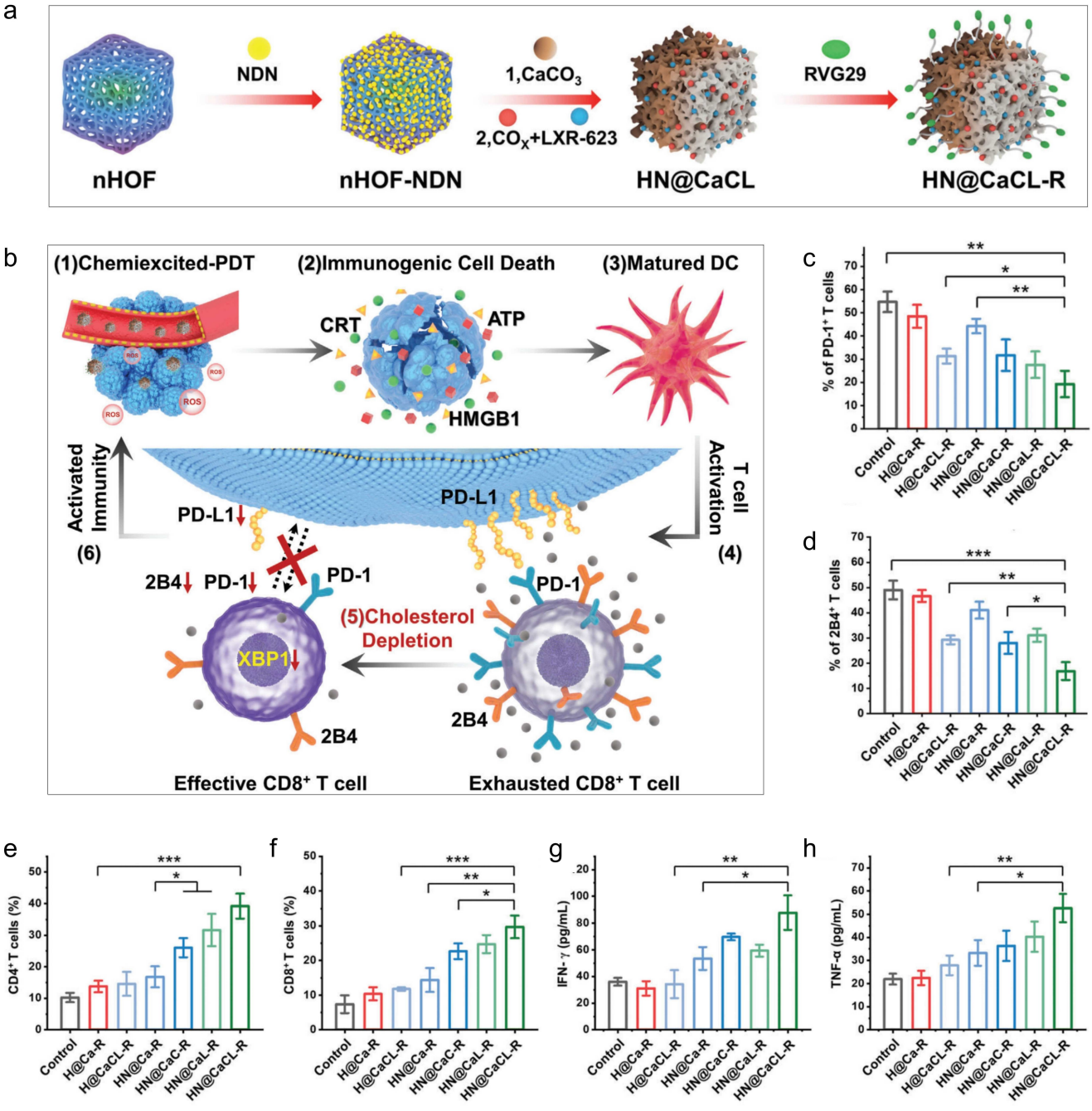


Figure 2. (a) Preparation of rabies virus glycoprotein 29 (RVG29) modified (nHOF-NSP-DMAE-NHS@$CaCO_3$-COX/LXR-623-RVG29, HN@CaCL-R) biotuners. (b) Proposed mechanism of the immune response and growth inhibition of orthotopic glioblastoma by HN@CaCL-R biotuners. (c,d) Expression of programmed cell death protein 1 (PD-1), and cluster of differentiation 244 (CD244, 2B4). Ratios of $CD4^+$ T cells (e) and $CD8^+$ T cells (f) in glioma tissues. Interferon-gamma receptor (IFN-γ) (g) and anti-tumor necrosis factor-α (TNF-α) (h) expression levels in glioma tissues ($n = 3$, *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; and ****, $p < 0.0001$).[31d] Copyright 2023, Wiley-VCH.

fluidics technology to formulate shDHCR7-NRs by blending the organic lipid components (CSP, PN, DOPE, and DSPE-PEG-MAN) with the aqueous shDHCR7 solution. The shDHCR7-NR formulations effectively curtailed cholesterol supply and reverted the immunosuppressive TME. Furthermore, a synergistic effect was observed when DHCR7 silencing was combined with toll-like receptor agonistic sequences (sRNA), promoting macrophage polarization with upregulation of interferon-gamma (IFN-γ) secretion and downregulation of interleukin 10 (IL-10) secretion and the mannose receptor cluster of differentiation 206 (CD206) expression (Figure 3b). Moreover, the regulation of cholesterol synthesis in GSMs inhibited $CD8^+$ T cell exhaustion and downregulated immune checkpoints such as PD-1, LAG3, and 2B4, while enhancing cytokine secretion. Treat-

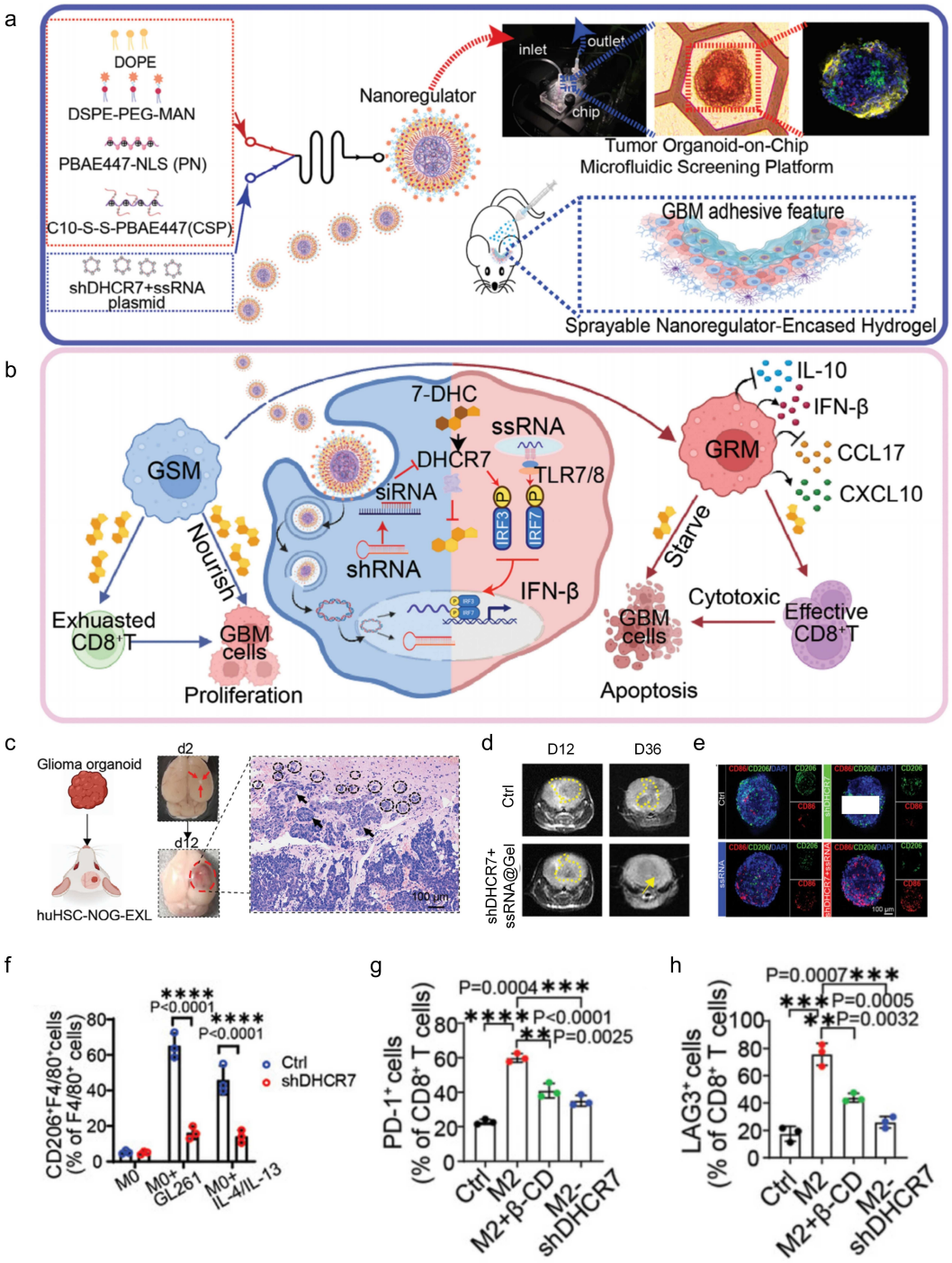


Figure 3. (a) The use of an intracavitary sprayable nanoregulator-encased hydrogel to modulate cholesterol metabolism in glioma-supportive macrophages (GSMs). (b) Improvement of the effectiveness of toll-like receptor (TLR7/8) agonists for glioblastoma (GBM) eradication. (c) Glioma biopsy organoids were transplanted into the cortex of huHSC-NOG-EXL mice using craniotomy. (d) T_2-weighted magnetic resonance imaging (MRI) images depict postoperative mice with glioblastoma organoid (GBO) xenografts following treatments. (e) Fluorescence images of GBO sections stained with anti-human CD86 antibody (red), anti-human CD206 antibody (green), and DAPI (blue) for various experimental groups. Scale bar: 100 μm. (f) M0-like bone marrow-derived macrophages (BMDMs) with 7-dehydrocholesterol reductase (DHCR7) silencing were cocultured with GL261 glioma cells or IL-4/IL-13 for 72 hours. The percentage of $CD206^+$ cells was assessed by flow cytometry ($n=3$). (g, h) Flow cytometry analysis of PD-1 (g) and LAG-3 (h) expression levels in $CD8^+$ T cells cocultured with different M2-like BMDMs ($n=3$). Data are displayed as the mean ± s.d. Statistical significance was calculated using an unpaired two-tailed student's t test (g,h) and two-way ANOVA (f). $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$.[34a] Copyright 2023, Wiley-VCH.

ment with shDHCR7 + ssRNA-NRs increased $CD8^+$ T cells and reduced $Foxp3^+$ Treg cells in tumors.

As the intracranial glioma model may not recapitulate the features of human GBM, the researchers implanted glioblastoma organoids (GBOs) into the brains of adult huHSC-NOG-EXL mice to emulate the heterogeneity of human GBM (Figure 3c). Tumor formation was evident 12 days post-GBO transplantation, characterized by numerous invasive lesions encompassing the original xenograft sites. The shDHCR7 + ssRNA-NRs combination treatment over 8 days effectively reduced the cholesterol levels in GBM tissues and tissue interstitial fluids to prolong the survival and slow the postoperative recurrence of GBO xenograft mice (Figure 3d). The combination treatment elevated the percentage of $CD68^+CD86^+$ GSMs while reducing the percentage of $CD68^+CD206^+$ GSMs in GBOs (Figure 3e). DHCR7 knockdown significantly impeded the M2 phenotypic polarization induced by tumor cells or IL-4/IL-13, suggesting that DHCR7 is essential for promoting macrophage polarization to an M2-like phenotype (Figure 3f). DHCR7 deficiency in M2-like bone marrow-derived macrophages (BMDMs) mitigated $CD8^+$ T cell exhaustion by reducing cholesterol supply, which effectively lowered the expression levels of PD-1 and LAG-3 (Figures 3g, 3h). Hence, the synergistic effect of intratumoral shDHCR7 and ssRNA therapy effectively improved the immunosuppressive TME and triggered systemic immune activation.

4. Mechanical Property Regulation

Cholesterol participates in steric interactions and hydrogen bonding through its 3β-hydroxyl group with other lipids and proteins.[35] In particular, the interactions of cholesterol with polar phospholipids lead to localized enhancements of lipid order in the membrane. As a result, cholesterol significantly contributes to the regulation of membrane mechanical properties such as fluidity, permeability, and rigidity.[36]

4.1. Mechanical Tension Regulation of Cancer Cell Membrane

Cell membrane mechanical tension, characterized as the force per unit length exerted on a cross-section of phospholipid bilayers, regulates a multitude of cellular processes including morphology, adhesion, migration, and cell division. Moreover, it impacts mechanosensitive ion channels and material transport to the cytoplasm.[27,37] Cell membranes exhibit resistance to mechanical tensions of up to $10^{-2}\,N\,m^{-1}$ under normal physiological conditions.[38] In response to external stimulations, cells employ several strategies to withstand mechanical strain. These include the formation of membrane invaginations/blebs, passive adjustment of cytoskeletal-membrane connections, and active regulation of mechanosensitive channels and membrane trafficking to prevent lysis.[39] Nevertheless, when the strain on membrane lipids surpasses a critical threshold, cells undergo physical rupture. While membrane pores smaller than a critical size can promptly reseal, they still compromise membrane stability. Interventions to modulate cell membrane tensions offer a new avenue to target cancer cells.

Lin and co-workers designed a CHO-functionalized Hf-TBP nMOF (Hf-TBP/CHO) to achieve synergistic anticancer effects via cholesterol depletion by CHO and PDT effect mediated by Hf-TBP.[40] Hf-TBP was synthesized via a solvothermal reaction between $HfCl_4$ and 5,10,15,20-tetra(p-benzoato)porphyrin (H_4TBP) in N,N-dimethylformamide with formic acid as the modulator at 125 °C for 2 days. Hf-TBP adopts MOF-545 structure and is comprised of $Hf_6(\mu_3\text{-}O)_4(\mu_3\text{-}OH)_4$ secondary building units (SBUs) and photosensitizing TBP ligands. CHO is adsorbed onto Hf-TBP to afford Hf-TBP/CHO (Figure 4a). Interestingly, Hf-TBP/CHO-treated cells exhibited an obvious decrease in F-actin assembly in the cell center and an increase of F-actin signal at the periphery (Figure 4b). In addition, Hf-TBP/CHO-treated cells showed an obvious influx of calcium ions in response to hypotonic solutions (Figure 4c), while no obvious difference was found in Hf-TBP-treated cells. In the meantime, Hf-TBP/CHO-treated cells significantly increased the cell membrane tension as probed by increased τ1 and τ2 lifetime values of fluorescent lipid tension reporter (Flipper-TR, Figures 4d–4g). Hf-TBP/CHO also increased the osmotic fragility of cancer cells to hypotonic solution, which increased rupturing propensity and induced influx of calcium ions for effective cancer therapy. As the PDT effect of Hf-TBP induced the pyroptosis of cancer cells characterized by caspase-1 mediated cleavage of gasdermin D (GSDMD), membrane rupture, and cellular swelling with large bubbles, elevated cell membrane tension enhanced cancer cell damage by PDT via reducing tolerance to ROS and increasing rupturing propensity of cancer cells. Subcutaneous 4T1 and MC38 tumors were established to evaluate the anti-tumor efficacy. Hf-TBP plus light irradiation slightly suppressed tumor growth with TGI indices of 54.3 % and 79.8 % for 4T1 and MC38 tumors, respectively (Figures 4h, 4i), while Hf-TBP/CHO plus light irradiation effectively inhibited the growth of 4T1 and MC38 tumors with TGI values of 91.7 % and 95.0 %, respectively.

4.2. Cytoskeleton and Metastasis

Invasion and metastasis stand as major challenges in cancer treatment. It is of great significance to develop therapeutic interventions for preventing or eliminating metastasis. As lamellipodia contribute to the invasion and metastasis of cancer cells by inducing changes in the actin cytoskeleton, research efforts have been devoted to inhibiting tumor metastasis by targeting lipid rafts and lamellipodia formation.[41] Wang and co-workers designed a multi-enzyme nanomedicine for anti-metastasis therapy by upregulating cellular oxidative stress and depleting cholesterol (Figure 5a),[29] They synthesized a mixed metal Zn/Co MOF with the 2-methylimidazole ligand and calcined the MOF in the presence of PPh_3 to obtain single-atom catalyst (Co-PN_3 SA) with the proposed Co-PN_3 coordination motif. Co-PN_3 SA catalyzed H_2O_2 decomposition to generate highly toxic •OH via a Fenton-like reaction. Co-PN_3 SA was then coated with

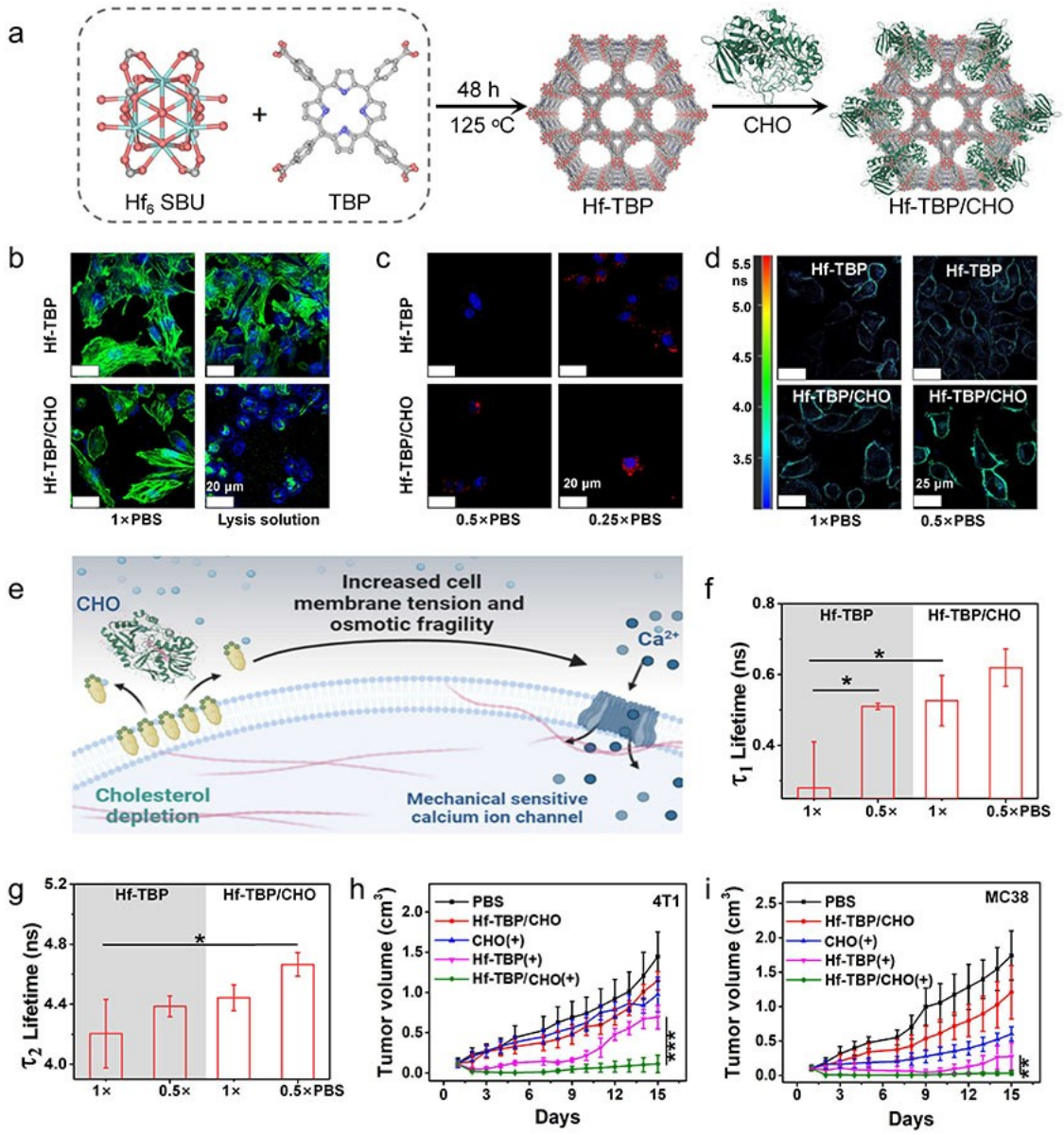


Figure 4. (a) Synthesis of Hf-TBP and Hf-TBP/cholesterol oxidase (CHO). (b) F-actin cytoskeleton assemblies of 4T1 cells pre-incubated with Hf-TBP or Hf-TBP/CHO (CHO: 20 pM) for 12 h followed by incubation with lysis solution for 1 min. (c) Calcium ion influx in 4T1 cancer cells after various treatments. (d) Assessment of mechanical tension of plasma membrane in 4T1 cells after different treatments as probed by fluorescent lipid tension reporter (Flipper-TR) lifetimes. (e) Schematic illustration of how cholesterol depletion leads to increased mechanical tension and osmotic fragility in cell membranes, resulting in calcium ion influx and increased rupture of cancer cells upon exposure to hypotonic solutions. (f,g) Analysis of τ1 and τ2 lifetimes of Flipper-TR in 4T1 cells after various treatments. Tumor volumes in 4T1 (h) and MC38 tumors (i) after different treatments. ($n=3$ for (f,g), and $n=5$ for (h,i); *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; and ****, $p<0.0001$).[40] Copyright 2023, Wiley-VCH.

CHO and bovine serum albumin (BSA) to form the final nanoplatform. Co-PN_3 SA efficiently converted H_2O_2 to O_2 at the tumor site, which then provides CHO the substrate to reduce cholesterol levels and generate more ROS.

As shown in Figures 5b, 5d–5e, Co-PN_3 SA/CHO depleted cholesterol to significantly affect lysosomal membrane integrity, caused mitochondrial membrane damage, and impeded cancer cell proliferation and migration by disrupting lipid rafts as confirmed by Filipin III and cholera toxin B subunit-FITC (CTB-FITC) staining. The impact of cholesterol depletion on lamellipodia formation was assessed by labeling fibros-actin (F-actin) stress fibers. Co-PN_3 SA/CHO treatment inhibited the formation of invasive lamellipodia (Figure 5c). The suppression of cell invasion and migration by Co-PN_3/CHO was further supported by wound healing assays (Figures 5f–5g). In vivo studies showed that the combination of CHO and Co-PN_3 SA also significantly decreased the expression of matrix metallopro-

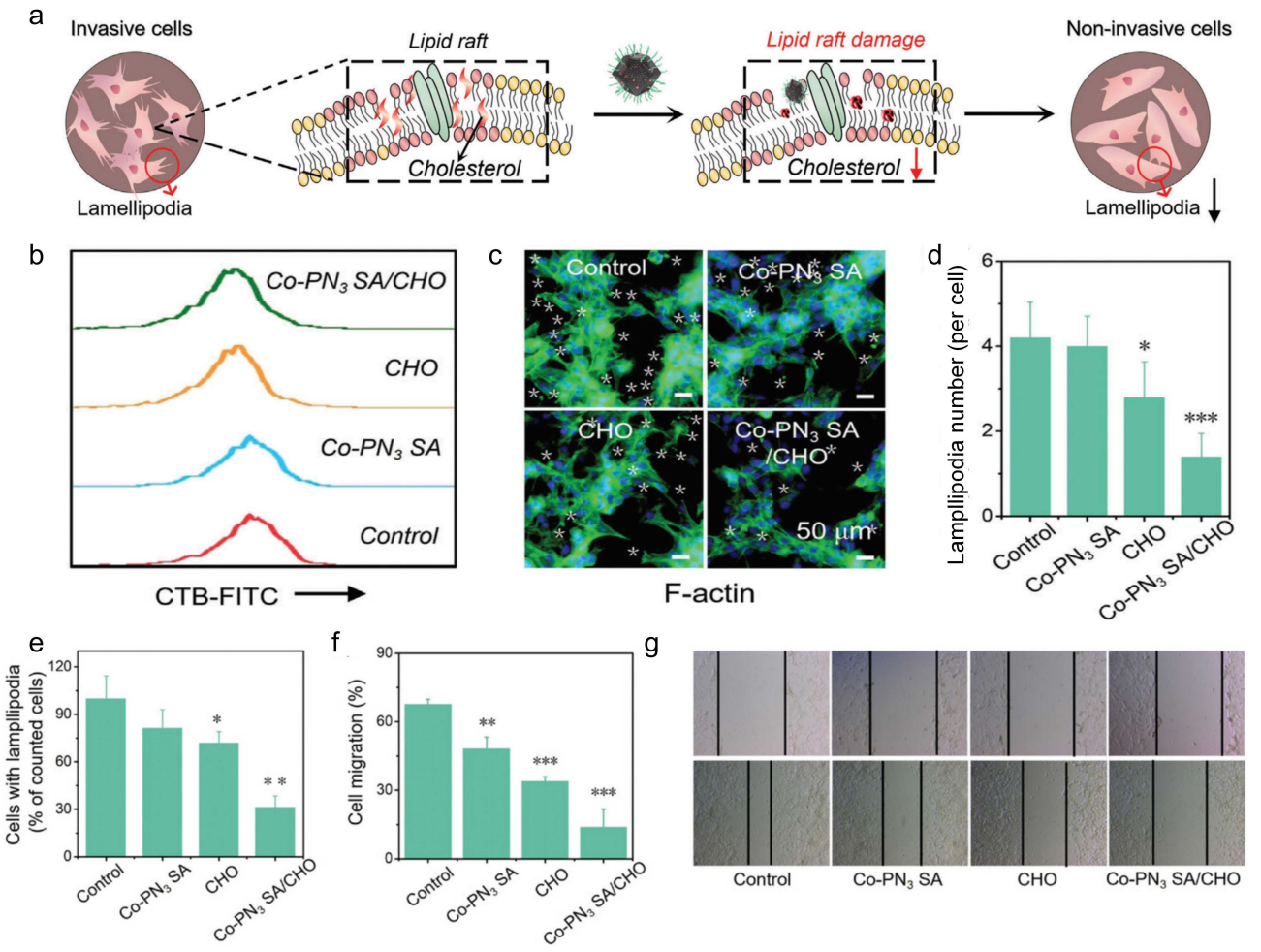


Figure 5. (a) Reduced tumor invasiveness by inhibiting lamellipodia formation and disrupting lipid raft integrity. (b) Assessment of lipid raft integrity using the cholera toxin B subunit-FITC (CTB-FITC) probe. (c) Immunofluorescence of F-actin and the alterations in lamellipodia formation (lamellipodia indicated by asterisks). (d,e) Quantitative analysis of lamellipodia per cell and the percentage of cells with lamellipodia among 4T1 cells. (f,g) The inhibitory effect of Co-PN_3 SA/CHO on cell invasion and migration assessed through wound healing assay.[29] (Data are means ± SD ($n = 3$). *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ by Student's two-tailed t-test.) Copyright 2024, Wiley-VCH.

teinase (MMP-9), which is linked to cell invasion and metastasis, and inhibited tumor metastasis.

5. Cell Metabolism Regulation

5.1. Reduced Chemoresistance

As a common cancer treatment, chemotherapy uses chemotherapeutics to kill fast-proliferating cancer cells and inhibit tumor growth. However, therapeutic effects of chemotherapy are limited by lack of specificity, moderate therapeutic indices, and multidrug resistance (MDR).[42] Elevated levels of cholesterol are commonly observed in the membranes of MDR cancer cells,[43] which serve as a barrier between the cell and its external environment.[44] This raises the question if cholesterol inhibition can be used to enhance chemotherapy and overcome MDR.[45] Extensive research efforts have focused on inhibiting transport protein expression to overcome drug resistance. For example, p-glycoproteins (P-gp),[46] the drug efflux transporter located in cholesterol-rich and drug-resistant cancer cell membranes, present a significant challenge in overcoming drug resistance stemming from this physiological barrier. As many chemotherapeutic drugs are P-gp substrates, including DOX, paclitaxel, cisplatin, and colchicine, high expression of P-gp prevents intracellular accumulation of chemotherapeutic drugs and leads to drug resistance.[47]

Zhou and co-workers used DOX as a representative drug to investigate the relationship between cholesterol level and resistance to conventional chemotherapeutics. They loaded DOX and CHO in the NH_2-MIL-88B MOF. The resulting DOX@MOF-CHO nanoparticles were further coated with a chondroitin sulfate gel shell (CS-shell) to enhance the sensitivity of tumor cells to chemotherapeutic drugs by endowing the particles with CD44 targeting and apoptosis-inducing properties via inhibiting the synthesis of the pro-inflammatory enzyme cyclooxygenase-2 (COX-2) and downregulating the expression of anti-apoptotic protein B-cell lymphoma-extra-large (BCL-XL). Elevated levels of

GSH in the TME cleaved the disulfide bonds of the CS shell to trigger the release of loaded DOX, initiating cascading catalytic reactions and apoptosis pathway to enhance the overall therapeutic effects. In addition, the DOX@MOF-CHO@CS nanosystem depleted cholesterol in drug-resistant cell membranes to reduce cell membrane fluidity and generate H_2O_2 for potent anticancer effects.

5.2. Cholesterol Metabolism and Anti-Tumor Immune Response

As a crucial lipid component, cholesterol contributes to the formation of effective immunological synapse characterized by a compact structure, closely linked to the granule and cytokine production of activated T cells. Previous research has shown that suppression of acyl coenzyme a-cholesterol acyltransferase-1 (ACAT-1) activity inhibits cholesterol esterification in T cells and enhances the antitumor immune function of T cells.[48] However, tumor cells tend to accelerate cholesterol biosynthesis[49] and uptake to sustain self-growth, metastasis, and invasion,[50] which allows them to compete with T cells for nutrients and generate metabolites for immunosuppression.[49] Because of the reliance of T cells and tumor cells on cholesterol metabolism,[51] significant efforts have been devoted to reprogramming the metabolic profiles of cholesterol in the TME.[52] Recently, statins, a family of well-known inhibitors of cholesterol biosynthesis through the MVA pathway, showed an unexpected function as vaccine adjuvants.[53] Lipophilic statins have shown efficacy in reducing the geranylgeranylation of the small GTPase Rab5, which enables enhanced antigen presentation and elicits antitumor responses.[54] Thus, the delivery of cholesterol metabolism regulatory drugs using engineered multifunctional nanoparticles has shown promise in regulating T cell signaling and enhancing cancer immunotherapy.[2b]

Li and co-workers developed a MMP-2-sensitive and tumor-penetrating nanovesicle to regulate cholesterol metabolism and enhance PDT and cancer immunotherapy. The EALP nanovesicles were synthesized by encapsulating an MMP-2-sensitive photosensitizer-conjugated peptide (PPa-PLGLAG-iRGD) and avasimibe (AVA) in a liposome. The photosensitizer pheophorbide A (PPa) was conjugated to internalizing RGD (iRGD, CRGDKGPDC) via an MMP-2-sensitive peptide linker (PLGLAG) to induce PDT activity and ICD (Figure 6a).[50] EALP had a cholesterol, PPa-PLGLAG-iRGD, and AVA molar ratio of 19.8:1.0:8.0:2.1:2 and AVA, PPa, and iRGD loading capacities of 3.52 %, 3.1 wt %, and 5.49 wt %, respectively. As a small molecule inhibitor of ACAT-1, AVA has been used to regulate cholesterol metabolism in the TME for cancer treatment. The tumor-penetrating peptide iRGD interacted with neuropilin-1 (Nrp-1) expressed on cancer cell membranes to facilitate transmembrane effects and enable deep tumor penetration (Figures 6b, 6c). EALP released 14.05 % and 28 % iRGD after incubation in MMP-2 rich buffer for 5 min or 2 h, respectively. This result showed that iRGD could be cleaved by overexpressed MMP-2 in the tumor tissue to promote deep tumor penetration. AVA disrupted cholesterol metabolism to revitalize cytotoxic T lymphocytes and maintain tumor cells under immune surveillance. The western blot analysis demonstrated reduced cholesterol metabolism after AVA or EALP treatment with downregulated expressions of SREBP2 and integrin (Figures 6d–6f). Thus, EALP treatment decreased plasma membrane cholesterol (Figures 6g–6i) to inhibit the migration of cancer cells and reduce tumor metastasis. EALP plus laser irradiation significantly inhibited tumor growth and increased median survival tumor-bearing mice by 21 days. This study presents a TME-responsive nanovesicle with tumor-penetrating capabilities to enhance PDT effect and cancer immunotherapy through dual regulation of cholesterol metabolism.[55]

6. Summary and Outlook

Cancer cells exhibit altered cholesterol metabolism to support their fast growth and survival whereas cholesterol metabolites produced by cancer cells exhibit immunomodulatory properties. Thus, cholesterol regulation is an emerging research area with promising implications in cancer therapy. Most research efforts have focused on exploring the potential anti-cancer effect of cholesterol-lowering drugs, including targeting cholesterol transporters, such as ATP-binding cassette (ABC) transporters and scavenger receptor class B type 1 (SR–B1), and modulating cholesterol synthesis pathways, for example, by targeting HMG-CoA reductase. In this minireview, we highlight recent advances in utilizing multifunctional nanomaterials to regulate cholesterol metabolism and enhance cancer therapy (Table 1). Conventional cholesterol-lowering drugs have a well-established track record in clinical use but lack the multifunctional capabilities of nanomaterials. Compared to conventional cholesterol-lowering drugs, multifunctional nanomaterials can be designed for controlled or sustained release of therapeutic agents, providing a more consistent therapeutic effect and reducing the frequency of dosing. They can be engineered to perform multiple functions, such as imaging, therapy, and treatment response monitoring, in a single system. This multifunctionality can potentially provide a more precise approach to manage cholesterol metabolism. In addition, nanomaterials may reduce the risk of off-target effects and lower general toxicity. Significant progresses on our fundamental understanding of cholesterol's roles on regulating cell death and the effective delivery of cholesterol regulating agents by nanomaterials are needed to ensure successful development of cholesterol-altering nanotherapeutic interventions for effective cancer therapy.

First, cholesterol is a vital component of cell membranes and is involved in various physiological processes throughout the body. Disruption of cholesterol metabolism may affect normal cells and tissues, potentially leading to off-target or systemic side effects.

Second, cholesterol homeostasis is governed by intricate feedback mechanisms with the involvement of many enzymes in cholesterol metabolism, cholesterol transporters, and other complicated regulatory factors. Targeting a single

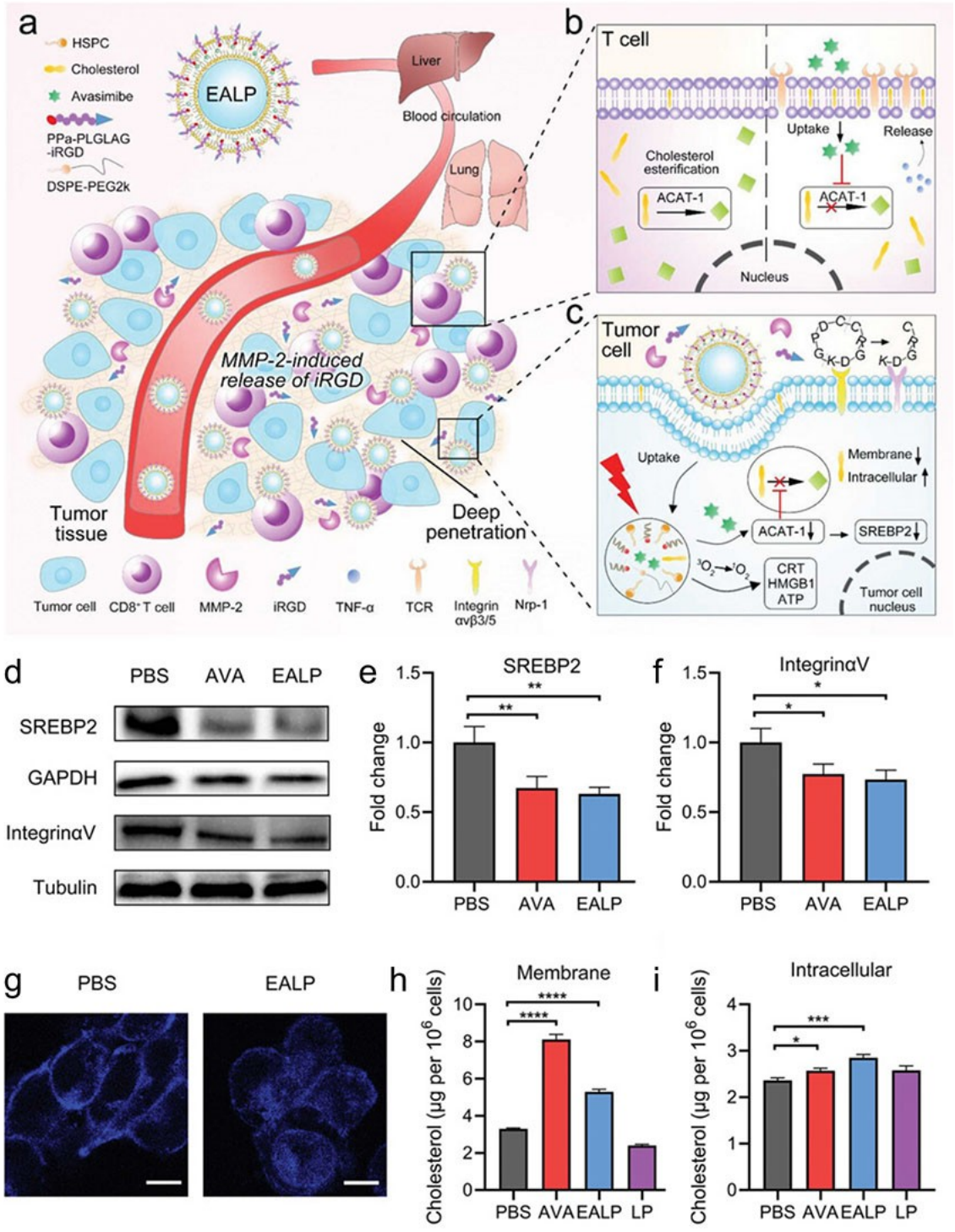


Figure 6. (a–c) Proposed therapeutic mechanism of matrix metalloproteinase (MMP-2) -sensitive EALP nanovesicles. (a) After *i. v.* injection, EALP accumulates in tumors and releases internalizing RGD (iRGD) in response to abundant MMP-2. The iRGD enhances nanovesicles and avasimibe (AVA) penetration into tumors. (b) EALP enhances T cell function by inhibiting cholesterol esterification. (c) EALP induced immunogenic cell death (ICD) by pheophorbide A (PPa)-mediated PDT. (d) western blot analysis and (e) semi-quantitative analysis of sterol regulatory element-binding proteins (SREBP2) and (f) Integrin αV in B16-F10 cells. (g) Cholesterol distribution in B16-F10 cells after EALP treatment as visualized by filipin III staining. (h) Membrane and (i) intracellular cholesterol levels of $CD8^+$ T cells treated with AVA or EALP. Data are means ± SD ($n = 3$). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.000$.[50] Copyright 2023, Wiley-VCH.

pathway of cholesterol homeostasis may have limited impact on tumor growth and disrupting any part of this pathway may have unintended effects on tumor cells and other cells in the TME. Tumor cells may develop resistance to

Table 1: Cholesterol regulation with multifunctional nanoplatforms.

Multifunctional platforms	Loaded drugs	Tumor model	TGI	Ref
DOX@CHO-ZrMOF(Cu)	DOX, CHO	MCF-7	94.4%	[18]
Cu-DBCO/CL	LOX-IN-3, CHO	4T1	87.2%	[19]
	Me-β-CD	B16F10	Eradication of 5/12 tumors, durable cures in 41.7% of treated mice	[27]
HN@CaCL-R	LXR-623, CHO, NDN, RVG29	GBM	–	[31d]
Sprayable nanoregulator encased hydrogel system	shDHCR7 (combined with sRNA TLR7/8 agonists)	GBM	–	[34a]
Hf-TBP/CHO	CHO	4T1, MC38	91.7% (4T1), 95% (MC38)	[40]
Co-PN_3 SA/CHO	CHO	4T1	Not provided, Figure 6d	[29]
EALP	AVA, PPa-PLGLAG-iRGD, PP	4T1	Not provided, Figure 5a	[50]

cholesterol metabolism inhibition by upregulating alternative pathways or acquiring mutations to render them less susceptible to cholesterol-altering interventions. A comprehensive understanding of intricate interplays between cholesterol metabolism and the TME will allow the development of therapeutic strategies that concurrently target cholesterol synthesis, uptake, esterification, or trafficking in cancer.

Third, the sizes, morphologies, structures, and charges of nanomaterials affect the loading capacities and the release of cholesterol regulating agents. Nanoparticles interact with various plasma proteins in circulation to affect their biodistribution properties. Vascular permeability and tumor cell endocytosis of nanotherapeutics further impact their anti-cancer effects.

In summary, deregulation of cholesterol balance plays a crucial role in cancer development. Cholesterol-altering multifunctional nanomaterials have been examined to regulate oxidative stress, modulate immune checkpoints, manipulate mechanical stress responses, and alter cholesterol metabolic pathways. However, the construction of cholesterol metabolism-altering nanoplatforms presents a bottleneck for advancing this strategy for cancer treatment. Nanocarriers with good stability, biocompatibility, high loading capacities are needed, and existing DDSs such as liposomes, solid lipid nanoparticles, nMOFs, and polymeric nanoparticles may not be ideal for functionalization with specific ligands or drugs to target cholesterol-related pathways. Two-dimensional and mesoporous nanomaterials may provide new avenues to deliver cholesterol metabolism-regulating molecules to tumors. Controlled or triggered release of these therapeutics from the nanoplatforms can further enhance their antitumor effects and reduce general toxicity. Therapeutic strategies targeting the following cholesterol metabolism regulation pathways can be considered: 1) Incorporating therapeutic entities to affect cholesterol synthesis, such as statins or other inhibitors of cholesterol biosynthesis; 2) Delivering therapeutic agents to enhance cholesterol efflux, such as apolipoprotein A1 mimetics or other transporters involved in cholesterol efflux; and 3) Designing nanoplatforms to interact with LDL receptors for cholesterol uptake modulation. We anticipate the development of efficient strategies to disrupt cholesterol metabolism and their synergistic combination with existing cancer therapies for effective cancer treatment. However, integration of multiple functionalities into a single nanoplatform presents significant challenges for large-scale production with acceptable batch-to-batch consistency, thus adding barriers to meeting regulatory requirements for clinical translation.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: cholesterol · tumor therapy · nanomaterials · tumor microenvironment · immune response · immune checkpoints

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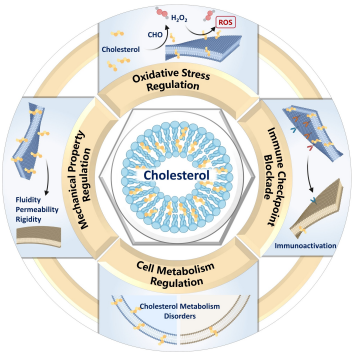
Minireview

Tumor Therapy

W. Zhen, T. Germanas, R. R. Weichselbaum, W. Lin* ——— **e202412844**

Multifunctional Nanomaterials Mediate Cholesterol Depletion for Cancer Treatment



In this minireview, we briefly discuss multiple roles of cholesterol in cancer progression, tumor microenvironment, and immune responses. We then summarize recent advances in cancer treatment through regulating cholesterol metabolism using multifunctional nanomaterials, via oxidative stress regulation, immune checkpoint modulation, mechanical stress response manipulation, and cholesterol metabolic pathway alteration.