Progress in Drug Delivery by Stimulus-responsive Liposomes in the Acidic Environment of Tumors

Yonghao Li *

Department of Pharmacy, Hebei University of Chinese medicine, Shijiazhuang, China

* Corresponding Author Email: yhl86571@gmail.com

Abstract. The tumor-associated microenvironment (TAM) being acidic is one of the significant characteristics of solid tumors. This affects the cellular uptake and activity of drugs, promotes the formation of drug resistance and invasiveness, and limits the therapeutic efficacy. Stimuli-responsive liposomes, compared with traditional drug carriers, have higher precision, utilization rate, and potential for combination therapy. They remain stable under physiological pH conditions but undergo structural changes or disintegrate in acidic environments, thereby achieving targeted drug release and enrichment. This offers broad application prospects in precise tumor treatment. The research on the acidic characteristics of the TAM has driven the development of various types of stimulus-responsive liposomes. pH-sensitive liposomes can achieve precise drug release in acidic environments; redox-type liposomes enhance drug release by taking advantage of the high ROS levels in tumors; enzyme-responsive liposomes rely on tumor-specific enzymes to trigger the dissociation of the structure. All three significantly improve the efficiency of drug delivery and therapeutic effects, providing multi-dimensional strategies and potential advantages for the design of anti-tumor nanomedicines. This review aims to systematically summarize the design strategies and application progress of stimulus-responsive liposomes in acidic tumor microenvironments, evaluate their *in vitro* and *in vivo* performance and clinical translational potential, and discuss the main challenges and corresponding solutions, with the expectation of promoting the clinical application of precise tumor treatment.

Keywords: TAM, stimuli-responsive liposomes, nanomedicine.

1. Introduction

The tumor microenvironment (TME) is a highly interactive network composed of tumor cells, fibroblast-like cells, immune cells (such as T cells, B cells, and tumor-associated myeloid cells), endothelial cells, and macrophages. These cells collaboratively secrete cytokines and remodel the extracellular matrix, collectively establishing an ecological niche that supports tumor growth, immune evasion, and metastasis [1]. Concurrently, the tumor site exhibits characteristic barriers, including elevated interstitial fluid pressure, excessive extracellular matrix (ECM) deposition, immunosuppression, and an acidic gradient. Among these, the acidic environment resulting from the Warburg effect poses a significant challenge to antitumor drug delivery [2]. The Warburg effect refers to the phenomenon wherein cancer cells predominantly metabolize glucose via glycolysis, producing large quantities of lactate even under oxygen-sufficient conditions, rather than relying on mitochondrial oxidative phosphorylation for energy. Another contributing factor to the acidic microenvironment is the conversion of lactate-derived pyruvate into carbon dioxide through the tricarboxylic acid cycle, which is subsequently transformed into carbonic acid within tumor cells, thereby releasing H^+ ions and exacerbating acidity [3]. This acidic milieu not only impairs normal immune cell function but also exacerbates the heterogeneity of drug penetration and distribution.

To address the dual challenge of insufficient drug concentration at tumor sites and systemic toxicity, nanomedicine delivery systems (NDDS) have emerged. NDDS can be designed to target tumor-specific markers and TME characteristics, thereby enhancing drug accumulation at the lesion site and prolonging circulation time [4]. To date, one of the most clinically successful NDDS platforms is liposomes (Lip), whose superior biocompatibility, low immunogenicity, high compatibility, and surface modifiability make them ideal carriers for antitumor drugs. Since the approval of doxorubicin liposomes in 1995, various liposomal formulations have become a new

therapeutic option for cancer patients. Among these, novel liposomes are categorized into three types: targeted modified liposomes, stimulus-responsive liposomes, and biomimetic liposomes [5].

Stimulus-responsive liposomes are a class of liposomes engineered with functional modules sensitive to endogenous or exogenous stimuli within the TME, enabling conformational changes or chemical bond cleavage under specific conditions to achieve controlled drug release and targeted delivery. Based on the stimulus source, they can be classified into endogenous and exogenous stimulus-responsive liposomes. Endogenous types include: (1) pH-sensitive liposomes, which respond to environmental pH variations via specific structural or functional components, currently constructed using low-pH insertion peptides or acid-sensitive phospholipids; (2) redox-sensitive liposomes, modified with cleavable bonds responsive to glutathione (GSH) and reactive oxygen species (ROS) in the TME; (3) enzyme-sensitive liposomes. Exogenous types encompass thermosensitive liposomes, photosensitive liposomes, and magnetic field-responsive liposomes, among others [6].

In acidic TME, pH-sensitive liposomes primarily employ two mechanisms for precise drug release. The first involves protonatable insertion peptides or acid-sensitive phospholipids that enhance membrane fusion at pH < 7.0, destabilizing the lipid bilayer to release payloads. The second strategy incorporates acid-labile chemical bonds that cleave under weakly acidic conditions, rapidly releasing active drugs or prodrugs at target sites. Furthermore, multimodal systems are gaining traction, concurrently responding to composite stimuli such as pH and ROS to further improve delivery efficiency and therapeutic safety. This review systematically summarizes the design strategies and application advances of stimulus-responsive liposomes in acidic TME, evaluates there *in vitro* or *in vivo* performance and clinical translation potential, and discusses key challenges and mitigation strategies to facilitate their clinical implementation in precision oncology.

2. pH-sensitive Liposomes

In the tumor microenvironment, cellular metabolism shows enhanced anaerobic glycolysis and accumulation of acidic metabolic products, both of which jointly cause a decrease in the pH value at the lesion site, making it acidic. The lowest extracellular pH value can reach 5.7. Based on this pathological feature, functional peptides that can be inserted can be introduced on the surface of liposomes, and an acid-sensitive scaffold can be selected to enable them to undergo conformational changes in an acidic environment, targeting and locking onto the acidic parts of the tumor, thereby constructing pH-sensitive liposomes that can selectively release and unfold at the tumor site. Currently, the main pathways for constructing pH-sensitive liposomes include using low pH insertion peptides/prefusogenic peptides for modification, or embedding molecules with specific acid-sensitive structures in the liposomes to regulate membrane stability [4].

Fusogenic peptide is also known as low pH low insert peptides (pHLIPs), which is a specific peptide carrier that responds specifically to the extracellular targeting characteristics of tumor cells. By modifying this peptide carrier onto the liposomes, the liposomes acquire the characteristic of responding to the pH environment. pHLIPs consist of flanking sequences and transmembrane sequences. The flanking sequences have multiple acidic-responsing residues distributed in the transmembrane region and insertion end to form the flanking sequences, while hydrophobic residues form the transmembrane sequences. The response peptides can undergo conformational responses to different pH values, presenting an irregularly coiled state or being distributed on the surface of the liposomes in the neutral environment, and in acidic conditions, the response peptides undergo coiling and form a helical conformation throughout the cell membrane. Based on the conformation under acidic conditions, pHLIPs can target and transport substances to the pathological tissues. Imaging agents and therapeutic drugs can be attached to the C-terminus of the membrane interior and the N-terminus of the membrane exterior and be targeted for drug delivery. Overall, its insertion mechanism is triggered by the protonation of negatively charged peptides under low pH conditions, which leads to an increase in the hydrophobicity of the peptide, thereby changing the distribution equilibrium of

the peptide in the bilayer. Liposomes modified with low pH insertion peptides have biocompatibility and can be used as a responsive drug delivery system to target tumors and enhance the absorption of cancer cells [7]. Combining the pH-sensitive phospholipid scaffold with specific molecular structures to construct a responsive entity that is sensitive to acidic environments. Phosphatidylethanolamine (PE0) as a pH-responsive phospholipid is a good basic carrier, often used in water-in-oil liposomes due to the intermolecular forces generated by its smaller group structure. Under physiological pH conditions, negatively charged small molecules with protonated acids combine with phosphatidylethanolamine to form liposomes with a special structure, and the surface repulsion formed by the negative charge stabilizes the new carrier. After stabilization, the acidic environment causes the liposomes to restore a hexagonal structure through the hexagonal crystal form of the amphiphilic carboxyl groups, resulting in regular instability and aggregation, and fusing and lysing with the cell membrane to release the contents [8]. Further, introducing PEG or ligands (such as RGD, folic acid) on the outer layer of the acid-sensitive scaffold can achieve long circulation and active targeting, thereby enhancing the selective enrichment and therapeutic effect at the tumor site in complex *in vivo* environments.

3. Redox-sensitive Liposomes

In the tumor-specific redox microenvironment, excessive GSH and ROS can both serve as stimuli for intelligent-responsive nano delivery systems. Using these factors, redox-sensitive small molecule prodrug nanoparticles can be designed. The preparation of liposomes using materials containing redox-sensitive bonds is one of the most common methods for introducing redox-sensitive bonds. Common redox-sensitive linkers include disulfide bonds, thioether bonds, diselenide bonds, selenoether bonds, thioketone bonds, and succinimide-sulfide bonds, among which disulfide bonds and thioether bonds are more widely used [9].

Disulfide bonds are commonly used chemical bonds for constructing redox systems. Disulfide bond complexes are stable in the body circulation and are oxidized by ROS when transported to the tumor pathological site, triggering the disintegration of the drug delivery system and the release of drugs. Chen et al. used the film dispersion method to dissolve lipid materials in organic solvents to form a monolayer lipid film, then transferred it to the aqueous phase. Through physical means such as vigorous stirring and ultrasound, the liposomes in the aqueous phase were broken and reformed into liposomes, synthesizing an oxidatively sensitive cationic oligopeptide lipid carrier. This system was used for combined delivery of paclitaxel and small interfering RNA (siRNA), significantly enhancing the anti-tumor effect and effectively overcoming the drug resistance caused by paclitaxel [10].

Thioether bonds are another type of redox chemical bond in the tumor acidic environment. In a high ROS environment, thioether bonds are easily oxidized to thiosulfone, causing the linker to change from hydrophobic to hydrophilic, thereby promoting the disintegration of the liposomes and the release of drug molecules. Wang et al. attached thioether bonds to polyethylene glycol to form a thioether bond polyethylene glycol that responds to ROS. They used it for coupling with hydrophobic near-infrared dyes and constructed nanoparticles with consistent particle size [11]. In summary, both disulfide bonds and thioether bonds will be oxidized to thiosulfone in a high ROS environment, significantly increasing the hydrophilicity of the molecules and accelerating the hydrolysis of the prodrug and the release of the drugs. These liposomes utilize the tumor-specific redox state to achieve controlled drug delivery effects and show promising clinical application prospects.

4. Enzyme-sensitive Liposomes

Enzymes are key factors for biochemical reactions in organisms, and in the microenvironment of tumor lesions and within tumor cells, the expression of many enzymes is upregulated, giving the tumor pathological site the ability to be targeted. Studies have shown that various enzymes such as

phospholipase C, phospholipase A2, alkaline phosphatase, and metalloproteinases are highly expressed in tumor or inflammatory sites, and these enzymes not only participate in the remodeling of extracellular matrix and signal transduction but also change the local chemical environment. Therefore, these enzyme sites can be used as a direction for constructing enzyme-sensitive liposomes [4]. Based on this, researchers propose to introduce structures that can be recognized by specific enzymes on the surface of liposomes or in the linkers to achieve precise release of drugs at the lesion site.

Liposomes improve stability and prolong circulation time through polymer coatings. This coating can be degraded by specific enzymes at the target site. The enzymatic cleavage of the polymer causes the instability of the liposomes and the release of the contents. Enzymatic liposomes can also carry ligands, further enhancing targeted delivery. In addition to the conventional passive release, lipid nano precursors in enzymatic delivery also have significant importance. In this method, the liposomes contain drug molecules chemically bound to the bilayer lipids. At the target site, the linker is cleaved by enzymes, and the active drug is released from the liposomes. Common enzyme-sensitive linkers include peptides recognizable by MMPs (such as GPLGVR), fatty acid ester bonds that can be cleaved by PLA_2, and phosphate groups that can be hydrolyzed under the action of alkaline phosphatase. The drug molecules are coupled to the lipid bilayer through these linkers, remaining stable in the blood circulation and being released efficiently at the target site due to enzymatic cleavage.

Enzyme-sensitive prodrug liposomes have significant advantages in delivering certain highly toxic and highly hydrophobic drugs [12]. These drugs often cannot achieve effective payloads through conventional physical encapsulation methods, but through chemical coupling and combination with enzyme-sensitive linkers, the drug loading can be significantly increased and non-specific toxicity can be reduced. Driven by enzymatic cleavage, the active drug is released controllably in the tumor microenvironment, thereby improving treatment efficiency and reducing damage to healthy tissues. Moreover, with the development of synthetic chemistry and bioengineering, new intelligent linkers are constantly emerging, such as multi-enzyme responsive structures or dual sensitivity designs (pH/enzyme dual sensitivity), providing new possibilities for improving the precision and stability of liposomes in complex *in vivo* environments.

5. Conclusion

TME is composed of various cells and extracellular components, featuring unique physicochemical characteristics such as acidity, hypoxia, and abnormal enzyme activity. These factors not only affect tumor growth and metastasis but also weaken the efficacy of traditional chemotherapy drugs. NDDS demonstrates significant advantages in tumor treatment by prolonging drug circulation time, enhancing tumor targeting, and controlling release performance. Stimuli-responsive liposomes based on TME abnormal characteristics, such as pH-sensitive, redox-type, and enzyme-responsive liposomes, can trigger drug release in specific environments, significantly increasing local drug concentration and therapeutic effect in tumors. These designs significantly enhance the targeting and therapeutic efficacy of nanomedicines, providing multi-dimensional strategies to overcome the limitations of traditional chemotherapy. pH-sensitive, redox-type, and enzyme-responsive liposomes all show good potential for targeted drug release, but they still have certain limitations. Due to individual differences and the different acidity levels of tumors and normal tissues in different locations, the response accuracy and circulation stability of pH-sensitive liposomes are limited. Redox-type liposomes rely on the ROS level in tumors, but their spatial distribution is uneven and the synthesis process is complex. Enzyme-responsive liposomes are limited by the heterogeneity and insufficient specificity of enzyme expression, and there is a risk of off-targeting. These issues collectively restrict the clinical translation and widespread application of these nanocarriers.

In tumor treatment, PPI therapy alters the pH gradient of cells to make cancer cells sensitive to chemotherapy drugs. At the same time, ROS plays an early role in PPI and the combination of PPI

and redox-type liposomes has a synergistic effect. In an environment rich in ROS, PPI can improve the acidic microenvironment, further promoting the release of redox-type liposome drugs. The drug delivery system of redox-type liposomes can precisely release drugs in the high-ROS state, increasing the drug uptake rate of tumor cells. Combined with the effect of PPI, this combined application may significantly improve the efficacy of drugs. In the future, the development of stimuli-responsive liposomes can focus on multiple response integration, intelligent regulation, and individualized design. By combining pH, redox, and enzyme response mechanisms, the precision of triggering and drug release efficiency can be improved; using new materials and biomimetic modifications can enhance circulation stability and tumor penetration ability; and combining molecular imaging and individualized tumor feature analysis, it is expected to achieve real-time monitoring and precise treatment, thereby promoting its clinical translation.

References

[1] Xiao Y, Yu D. Tumor microenvironment as a therapeutic target in cancer. Pharmacology & Therapeutics, 2021, 221: 107753.

[2] Chuan H, Yujun S, Huile G, et al. Research Progress on Responsive Nanodelivery Systems for Tumor Microenvironment in Tumor Immunotherapy. Progress in Pharmaceutical Sciences, 2022, 46 (7): 485 - 494.

[3] Ohaegbulam K C, Assal A, Lazar-Molnar E, et al. Human cancer immunotherapy with antibodies to the PD-1 and PD-L1 pathway. Trends in Molecular Medicine, 2015, 21 (1): 24 - 33.

[4] Xuerong D, Lihua J. Advances of New Liposomes in Anti-Tumor Drug Research. Journal of Clinical Drug Therapy, 2025, 23 (3): 11 - 18.

[5] Izadiyan Z, Misran M, Kalantari K, et al. Advancements in liposomal nanomedicines: Innovative formulations, therapeutic applications, and future directions in precision medicine. International Journal of Nanomedicine, 2025: 1213 - 1262.

[6] Xinhu G, Wentao X. Construction Strategies of Stimuli-Responsive Liposomes and Their Biological Applications. Journal of Biotechnology Engineering, 2024, 40 (6): 1742 - 1751.

[7] Junjuan F, et al. Research on the Role of PD-L1-Acid Responsive Peptide Combined with Avelumab in Stimulating ADCC Effect for Killing Tumors. 2024.

[8] Zhizhong Y, Zi-ting W, Peng W, et al. Application of Stimuli-Responsive Liposomes in Targeted Drug Delivery Systems. Chinese Journal of Biochemistry and Molecular Biology, 2020, 36 (12): 1395 - 1403.

[9] Yao Z, Canyu Y, Qiang Z, et al. Research Progress on Metabolism and Efficacy of Responsive Small Molecule Prodrug Nanoparticles in Tumor Redox Microenvironment. Acta Pharmaceutica Sinica, 2021, 56 (2): 476 - 486.

[10] Chen X, Zhang Y, Tang C, et al. Co-delivery of paclitaxel and anti-survivin siRNA via redox-sensitive oligopeptide liposomes for the synergistic treatment of breast cancer and metastasis. International Journal of Pharmaceutics, 2017, 529 (1 - 2): 102 - 115.

[11] Wang J, Sun X, Mao W, et al. Tumor redox heterogeneity-responsive prodrug nanocapsules for cancer chemotherapy. Advanced Materials, 2013, 25 (27): 3670 - 3676.

[12] Fouladi F, Steffen K J, Mallik S, et al. Enzyme-responsive liposomes for the delivery of anticancer drugs. Bioconjugate Chemistry, 2017, 28 (4): 857 - 868.