

Research progress in cyclopeptide: anticancer mechanisms and targeted delivery systems

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Abstract. Breast cancer is a serious health concern for women globally. Traditional therapies including surgery chemotherapy, radiotherapy and endocrine therapy can control breast cancer effectively, but their effectiveness is often hindered by drug resistance and side effects. Cyclopeptides, a class of biomolecules with a closed-loop structure, present a promising alternative due to their stability potency and specificity. This review delves into the mechanisms of cyclopeptides in breast cancer treatment, evaluating their effectiveness and challenges. And also discusses the studies concerning the anti-cancer activities and delivery systems of cyclopeptides such as cyclosporin A and deoxybouvardin (RA-V). Innovations in nanotechnology and targeted delivery systems further optimize cyclopeptide therapies, enhancing their efficiency and reducing side effects. Research suggests that cyclopeptides can effectively halt the proliferation and metastasis of breast cancer cells. They achieve this through various mechanisms, such as inhibiting key cancer pathways, inducing tumor cell apoptosis, and enhancing drug efficacy via targeted delivery systems. This review highlights the application of cyclopeptides in breast cancer treatment and discusses the targeted delivery technologies of cyclopeptides.

Keywords: Cyclopeptide, Breast cancer, Structure, Targeted delivery.

1. Introduction

Breast cancer continues to be a prevalent and deadly disease affecting women globally with alarming rates of both incidence and mortality. In 2022 statistics from the Global Cancer Observatory revealed that breast cancer makes up nearly a quarter of all new cancer cases among women with the highest mortality rate of 15.3% [1]. While traditional treatment options like surgery chemotherapy, radiation therapy and endocrine therapy have been effective to a certain extent, they are plagued by limitations such as reduced effectiveness drug resistance and severe side effects. Therefore there is an urgent need to explore and develop alternative treatment methods that can provide more effective and less harmful outcomes for breast cancer patients [2].

Amidst the challenges faced in breast cancer treatment, peptides, especially cyclopeptides, have risen as a promising solution because of their distinct pharmacological qualities. Peptides composed of two or more amino acids linked by peptide bonds offer a novel approach to combatting the disease [3]. However, while linear peptides have displayed great potential in breast cancer treatment, they encounter various obstacles. These hurdles include vulnerability to enzymatic breakdown, the requirement for frequent administration, restraint of the ability to pass through cell membranes and insufficient targeting capabilities [4]. These limitations greatly curtail the potential and efficacy of linear peptides as viable therapeutic options for breast cancer patients.

In contrast to linear peptides, cyclopeptides offer a promising solution to a variety of challenges. These cyclic structures formed by amino acid sequences that loop back on themselves present a more stable and effective option. There are four main types of cyclization methods: side-chain to side-chain head-to-tail head-to-side-chain and side-chain-to-tail [5,6]. By forming a circular structure, cyclopeptides decrease the flexibility of the peptide backbone and enormously reduce open ends. This makes them less susceptible to enzymatic degradation and enhances their stability, increasing affinity to receptors and overall biological activity [6,7]. This allows cyclopeptides to effectively target specific cancer cell receptors, thereby showing greater potential in cancer treatment. For example,

cyclopeptides can inhibit the transcription of cancer genes and thus affect the growth of cancer cells, block common signaling pathways in cancer cells to induce apoptosis, and participate in the formulation of drug delivery systems [8-11].

Although research on cyclopeptides is very active and continues to yield results, there is currently a lack of a systematic summary, especially regarding their anticancer mechanisms and targeted delivery technologies. Therefore, the main purpose of this article is to summarize recent advances in the research on the anticancer mechanisms of cyclopeptides and the development of cyclopeptide-targeted delivery systems. By thoroughly reviewing and analyzing the current scientific literature, this review aims to provide a comprehensive theoretical basis and framework for future research and applications of cyclopeptides in cancer treatment. It is hoped that this review will inspire more scientific interest and clinical exploration, further advancing the role of cyclopeptides in the treatment of breast cancer.

2. Mechanisms of anticancer activity of cyclopeptides

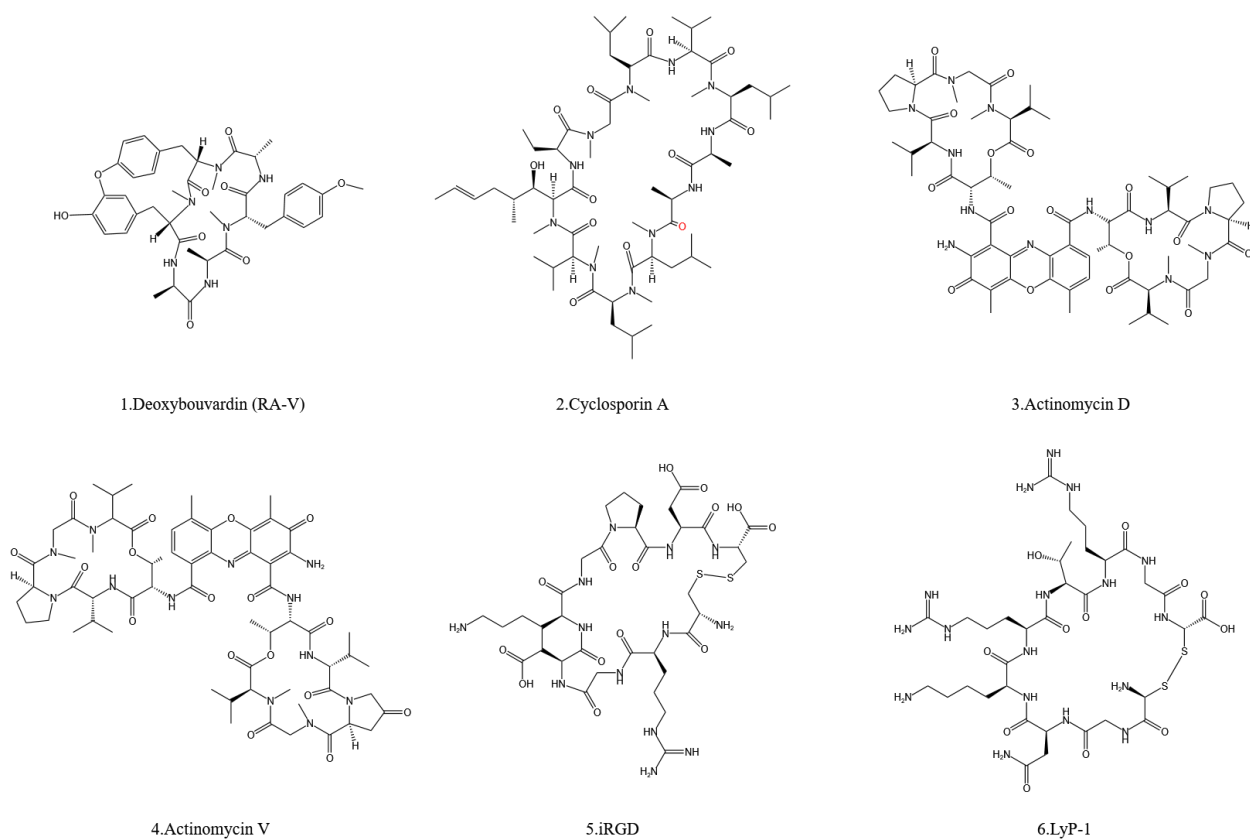


Figure 1. Examples of representative cyclopeptides.

Table 1. Comparing six cyclopeptides in breast cancer treatment

Property/ Compound	RA-V	CsA	Actinomycin D	Actinomycin V	iRGD	LyP-1
Anticancer effect	Effectively inhibit the growth of breast cancer cells	Inhibition of PKM2 expression in breast cancer cells	Increases apoptosis rate, inhibits degradation of P53	Compared to actinomycin D, IC50 is lower, improving cell adhesion	Enhances anticancer efficacy of PROTACs, increases penetration	Significantly improves targeting of imaging and treatment
Mechanism of action	Blocks PDK1-AKT interaction inducing apoptosis	Inhibits transcription of PKM2 gene, reducing ATP synthesis	Directly binds to the MDM2 oncogene, blocking signal transmission	Reduces expression of N-cadherin, decreasing cell migration	Mediates drug invasion into tumors through CendR motif interacting with NRP-1	Targets through p32 receptor, enhancing drug and imaging agent delivery
Selectivity	High selectivity for breast cancer cells	High specificity for cancer cells	Selective binding to high-CG content DNA	Reduces cell adhesion, inhibits migration and invasion	High tissue- specific penetration characteristics	High selectivity for tumor cells expressing p32

2.1. Plant cyclopeptides

RA-V, a cyclic hexapeptide extracted from the roots of *Rubia cordifolia*, exhibits significant inhibitory activity against both murine breast cancer cells (4T1 cells) and human breast cancer cells (MCF-7, MDA-MB-231) [10,12]. In human breast cancer cells (MCF-7, MDA-MB-231), RA-V disrupts chemokine receptor and serine protease signaling pathways involved in cell migration, thereby inhibiting the migration of breast cancer cells [13]. This cyclopeptide induces mitochondrial-mediated apoptosis by blocking the PDK1-AKT interaction, characterized by the loss of mitochondrial membrane potential, the release of cytochrome c, and the activation of the caspase cascade [10,14]. Moreover, RA-V reduces the expression of intercellular adhesion molecules, focal adhesion kinase, integrins, and vascular cell adhesion molecules, further inhibiting the adhesion of breast cancer cells [13]. The potential therapeutic effects of the plant cyclopeptide RA-V against breast cancer, particularly its mechanisms of inhibiting tumor cell growth and migration, provide a significant therapeutic target. By interfering with cell migration-related signaling pathways and inducing apoptosis, RA-V may become one of the effective drugs for future breast cancer treatments.

2.2. Microbial cyclopeptides

2.2.1. Fungal cyclopeptides

Cyclosporine A (CsA) is an eleven-amino acid cyclopeptide known for its potent immunosuppressive properties and also possesses anticancer characteristics. Compared to normal breast cells, pyruvate kinase M2 (PKM2) is overexpressed in MCF-7, MDA-MB-435, and MDA-MB-231 breast cancer cell lines. CsA can significantly reduce PKM2 production in these breast cancer cells by inhibiting the transcription of the PKM2 gene, thereby decreasing ATP synthesis and inducing cell death in breast cancer cells [9]. The anticancer effects of this drug may extend beyond the inhibition of PKM2, potentially involving other crucial cellular biological processes. In recent research, a targeted drug delivery system using CsA encapsulated in chitosan by hyaluronic acid nanoparticles has been developed to enhance the efficacy of CsA against breast cancer [10]. This targeted delivery technique using hyaluronic acid nanoparticles enhances the local concentration of the drug within tumor tissues, reduces side effects, and helps overcome drug resistance in tumor cells, thus enhancing the efficacy of CsA. Therefore, the study of CsA and its targeted delivery techniques

is significant not only for breast cancer treatment but also provides insights into the role of immunomodulators in cancer therapy.

2.2.2. Bacterial cyclopeptides

Actinomycin D, a chromopeptide naturally produced by *Streptomyces*, binds to DNA with high CG content [15]. Yang et al. explored the treatment effects of actinomycin D alone, doxorubicin alone, and their combination on triple-negative breast cancer. They found that the combination treatment resulted in higher rates of cell apoptosis. Actinomycin D can directly bind to the MDM2 oncogene to inhibit its transcription, also preventing the interaction between p53 and MDM2 which inhibits the degradation of p53, thus inducing apoptosis in triple-negative breast cancer cells [8]. Differently, Das et al. investigated the impact of actinomycin D on SOX2 expression in breast cancer cells. They discovered that Actinomycin D could specifically reduce the expression of the transcription factor SOX2, leading to the depletion of the breast cancer stem cell population [16]. In the breast cancer cell line (MCF-7 and MDA-MB-231), cells with high SOX2 have been confirmed to show greater migration capabilities, invasion and metabolic activity [17]. While actinomycin D has shown potential therapeutic effects in breast cancer treatment, a deeper understanding of its mechanisms of action and combined use with other therapies is still needed.

Actinomycin V, produced by marine *Streptomyces*, is an analog of actinomycin D, where prolyl-4-ketone replaces proline-4 in actinomycin D. Lin et al. showed that actinomycin V has a much lower IC₅₀ against MDA-MB-231 cell lines compared to actinomycin D, and treatment with actinomycin V reduced N-cadherin expression, improving cell-cell adhesion and reducing migration and invasion, particularly noted in MDA-MB-231 cells [18,19]. These studies underline the need for a deeper understanding of the mechanism of actinomycin V. Although anticancer effects and impacts on adhesiveness have been observed, the specific molecular mechanisms remain to be fully elucidated. Further experiments and research could help us better understand the action of actinomycin V, providing a solid foundation for its potential clinical application.

2.3. Synthetic cyclopeptides

Internalizing Arginine-Glycine-Aspartic acid (iRGD) is a cyclopeptide consisting of nine amino acids, demonstrating specific penetration abilities in tumor vasculature and tissues [20]. It effectively penetrates tumor tissues, spreading within them while minimizing damage to normal cells, and plays roles in targeted drug delivery and imaging. In innovative cancer treatment research, iRGD was conjugated with proteolysis targeting chimeras (PROTACs), leveraging its penetration properties to deliver PROTACs deep into breast cancer tissues. This enhanced the solubility, tissue selectivity, and penetration of PROTACs within tumor environments, thereby improving their anticancer efficacy [21]. This approach significantly minimizes drug exposure to non-target tissues, potentially reducing side effects.

Moreover, iRGD can also be doubly modified with a pH-responsive polymer, poly(2-ethyl-2-oxazoline), to target a novel drug delivery carrier made of mesoporous silica. The role of iRGD is to target the therapy, while the poly(2-ethyl-2-oxazoline) responds to the acidic environment within the tumor to facilitate drug release [22]. Both methods employ the RGD sequence to specifically bind to integrin $\alpha v \beta 3$ on the tumor surface, then through enzymatic cleavage, expose the C-end CendR motif. This motif interacts with NRP-1, a highly expressed transmembrane protein on tumor cells, facilitating the entry of iRGD and drugs into the tumor [23]. The dual modification with iRGD and the pH-responsive polymer utilizes the acidic tumor microenvironment for therapy. This specificity ensures drug release near tumor cells and reduces the impact on healthy cells, highlighting the precision of tumor-specific targeting in improving therapeutic outcomes. Future research should focus on optimizing the design and functionality of these iRGD-modified delivery systems to further enhance their specificity and efficiency. Clinical trials are necessary to validate these preclinical findings and explore the potential of iRGD in combination therapies.

LyP-1 is a cyclopeptide composed of nine amino acids that binds to the p32 receptor [24]. It is extensively used in breast cancer treatment and diagnostics, including as an imaging tool for triple-

negative breast cancer, in combination with nanomaterials for tumor treatment, and in LyP-1-modified oncolytic virus therapy. A Tc-labeled LyP-1 was used to analyze its targeting capabilities and immunofluorescence was utilized to evaluate the imaging effects of LyP-1 linked with fluorescein isothiocyanate (FITC) in tumors, significant for diagnosing triple-negative breast cancer [25]. Although LyP-1's targeting and imaging capabilities have been confirmed, a deeper understanding of its interaction with the p32 protein is necessary. Detailed investigation into how LyP-1 influences cancer cell signaling pathways could reveal its mechanisms in inhibiting tumor growth and metastasis, which is crucial for developing new therapeutic strategies.

Based on high expression of the LyP-1 receptor (p32) on the surface of breast cancer cells, Xu et al. constructed oncolytic viruses modified with LyP-1 (AdLyp.sT and mHAdLyp.sT), comparing them to control groups. LyP-1 improved the viruses' binding, replication, and transgene expression. AdLyp.sT and mHAdLyp.sT effectively suppressed tumor growth and bone metastasis in a human triple-negative breast cancer mouse model [26]. Given LyP-1's high selectivity for breast cancer cells, future research might explore its use as part of personalized treatment strategies. By tailoring treatment plans for individual patients, LyP-1 and its derivatives could offer more effective treatment options, particularly for cases that respond poorly to conventional therapies.

As LyP-1 treatment strategies advance, Zhang et al. developed a nanodevice with good colloidal stability, pH-regulated drug release, and high photothermal conversion efficiency. This device which mediated by CuS photothermal therapy and 5,6-Dimethylantraquinone 4-acetic acid, leverages LyP-1 to target and kill breast cancer cells while also triggering an anti-tumor immune response. Relying on LyP-1-mediated apoptosis, the nanodevice can eradicate lymphatic endothelial cells within the tumor. By concurrently disrupting both blood and lymphatic vessels, the device cuts off major tumor metastasis pathways, significantly impacting lung metastasis suppression in breast cancer models [27]. Although the design of the LyP-1 nanodevice shows promising therapeutic prospects, there is still room for improvement. For instance, further refining drug release mechanisms and enhancing photothermal efficiency could significantly improve treatment specificity and reduce side effects, advancing its application in clinical trials.

3. Targeted delivery systems

3.1. Cyclopeptide-assisted delivery of chemotherapy drugs

In cancer treatment, chemotherapy drugs are used for their ability to kill rapidly proliferating cells [28]. However, the non-specificity of chemotherapy drugs can also harm normal tissue cells, significantly limiting their therapeutic efficacy [28]. To enhance the accumulation of chemotherapy drugs in tumor tissues and reduce damage to normal tissue cells, researchers have developed various targeted drug delivery systems, with cyclopeptides playing a crucial role due to their targeting specificity and high stability.

LyP-1, a cyclic nonapeptide that specifically targets tumor cell surfaces and lymphatic endothelial cell surfaces via the p32 receptor, has been utilized in breast cancer research [24]. Timur et al. have developed a self-microemulsifying drug delivery system (SMEDDS) containing LyP-1 to deliver the chemotherapy drug doxorubicin hydrochloride. In vitro experiments showed that the LyP-1-modified SMEDDS system significantly enhanced the cytotoxic effects of doxorubicin hydrochloride on breast cancer cells compared to the control group. A 28-day in vivo study demonstrated that the combination of doxorubicin hydrochloride with the LyP-1-modified SMEDDS system significantly slowed the growth of breast cancer cells in mice and markedly reduced tumor cell metastasis and mouse mortality, further validating the effectiveness of the LyP-1-modified SMEDDS system for delivering chemotherapy drugs [29]. Considering these positive results, future research might explore optimizing the LyP-1 drug delivery system through different carrier systems and targeting molecules. This could include testing new biocompatible materials or developing more precise targeting mechanisms to enhance treatment outcomes and reduce potential side effects.

In the field of drug development, PROTACs represent a promising new strategy for drug development. As previously mentioned, facing limitations such as solubility, tissue selectivity, and penetration abilities of traditional PROTACs in tumor tissues, He et al. have conjugated the cyclopeptide iRGD with PROTACs. This conjugation effectively overcomes these deficiencies, enhancing the solubility, tissue selectivity, and penetration of PROTACs in tumor tissues, thereby improving their anticancer effects [21]. Nonetheless, further in-depth studies on the long-term safety and potential side effects of the iRGD-PROTACs conjugates are crucial to ensure the safety of these new therapies for successful clinical applications.

In recent years, Cell Penetrating Peptides (CPPs) have shown enormous potential for efficiently delivering drugs into cells, but they face challenges such as poor stability and low specificity [30]. To overcome these issues, cyclic Cell Penetrating Peptides (cCPPs) have emerged. cCPPs offer higher cell penetration, protease resistance, and specificity [30]. Park et al. have considered that cCPPs could compensate for the drawbacks of the drug cabazitaxel (CBT), known for its poor pharmacokinetics. They conjugated CBT with cCPPs using ester bonds and linked integrin-targeting peptides (RGDC, TP1) and fibronectin-targeting peptides (CTVRTSAD, TP2) through disulfide bonds. The results showed that compared to the control group, the conjugates TP1-cCPP-CBT and TP2-cCPP-CBT reduced the proliferative activity of integrin and EDB-Fn overexpressing cancer cell lines by about 3-4 times, but reduced the proliferative activity against normal tissue cells by 31-34 times [31]. Although this strategy reduced damage to cancer cell lines, it increased targeting specificity and environmental sensitivity, thereby better protecting normal tissue cells. This strategy could be expanded to develop composite conjugate systems containing multiple drugs to target several biomarkers of tumors, thus improving the comprehensiveness and efficiency of treatment.

3.2. How to deliver anticancer cyclopeptides?

The cyclic hexapeptide RA-V, as previously mentioned, has limited application in tumor treatment *in vivo* due to its low solubility under physiological conditions [12]. To address this issue, Qiao et al. have utilized a copolymer poly(β -amino ester) capable of loading RA-V. This copolymer can autonomously form micelle-like nanoparticles in a pH 7.4 aqueous solution. Loading RA-V into the hydrophobic core of the micelles not only enhances its solubility but also maintains stability under neutral conditions. Moreover, the carrier's acid-triggered copolymer ionization can accelerate drug release, exhibiting significant anticancer effects against breast cancer cells, and the micelles themselves are non-toxic [32]. This technique's successful application to RA-V provides a potential method to enhance the efficacy of other anticancer drugs with similar solubility issues. Applying this strategy to other drugs, especially those whose clinical applications are limited by low solubility, could open new therapeutic possibilities.

In research on triple-negative breast cancer, Maisa Siddiq Abduh developed a nanomedicine delivery technology, namely a CD44-targeted cyclosporine A-loaded thiolated chitosan nanoparticle formulation. Thiolated chitosan, obtained by introducing thiol groups into chitosan, enhances adhesion and penetration capabilities. As the surface ligand of the nanoparticles, hyaluronic acid can specifically bind to the CD44 receptor overexpressed on tumor cells. This nanomedicine effectively encapsulates cyclosporine A within thiolated chitosan through ionic gelation technology, protecting cyclosporine A from degradation and enabling controlled release upon reaching the target, enhancing treatment efficiency and reducing toxicity to normal cells [10]. To ensure the safety of long-term treatment, future research should focus on the biocompatibility and biodegradability of nanodelivery systems. Developing nanocarriers that can safely degrade in the body to avoid potential side effects from long-term accumulation, while maintaining effective drug release performance, is essential.

4. Conclusions and Prospects

This article reviews the application and progress of cyclopeptides in breast cancer treatment and discusses innovative developments in delivery systems for breast cancer research. Due to their unique closed-loop structure, cyclopeptides offer higher stability and selectivity than linear peptides, demonstrating substantial potential in anticancer therapy. Various types of cyclopeptides not only effectively inhibit tumor cell growth and migration but also induce tumor cell apoptosis through multiple mechanisms, including the suppression of key oncogene expression and blocking of critical cellular signaling pathways. Innovative nanomedicine delivery systems and self-microemulsifying systems utilize the high targeting and penetration capabilities of cyclopeptides, significantly improving therapeutic effects while reducing toxic impacts on healthy cells. The development of these systems has optimized drug distribution and release in the body, enhancing drug bioavailability and efficacy.

Despite significant progress, the application of cyclopeptides in breast cancer treatment still faces several challenges. The issues of large-scale synthesis and high costs need to be addressed to facilitate their broader clinical application. What's more, the effects and mechanisms of combining cyclopeptides with existing treatments require further exploration to optimize treatment regimens and enhance efficacy. To propel the field forward future investigations should focus on key areas of development. Firstly, efficient synthesis and production technologies must be developed to streamline the manufacturing process and reduce costs. Secondly, overcoming multidrug resistance in tumors is crucial, necessitating the creation of innovative drug delivery systems. Biocompatibility and safety concerns related to the use of nanomedicine delivery technologies also warrant thorough examination. Lastly, increased emphasis on preclinical and clinical trials is essential to evaluate the safety, efficacy and ideal usage of cyclopeptides and their delivery systems. It is anticipated that through dedicated research and development efforts in these areas cyclopeptides and their targeted delivery systems will revolutionize the landscape of breast cancer treatment, potentially extending their impact to a wider range of cancers in the future.

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