Targeting Mitochondria in Cancer Therapy: Machine Learning Analysis of Hyaluronic Acid-Based Drug Delivery Systems

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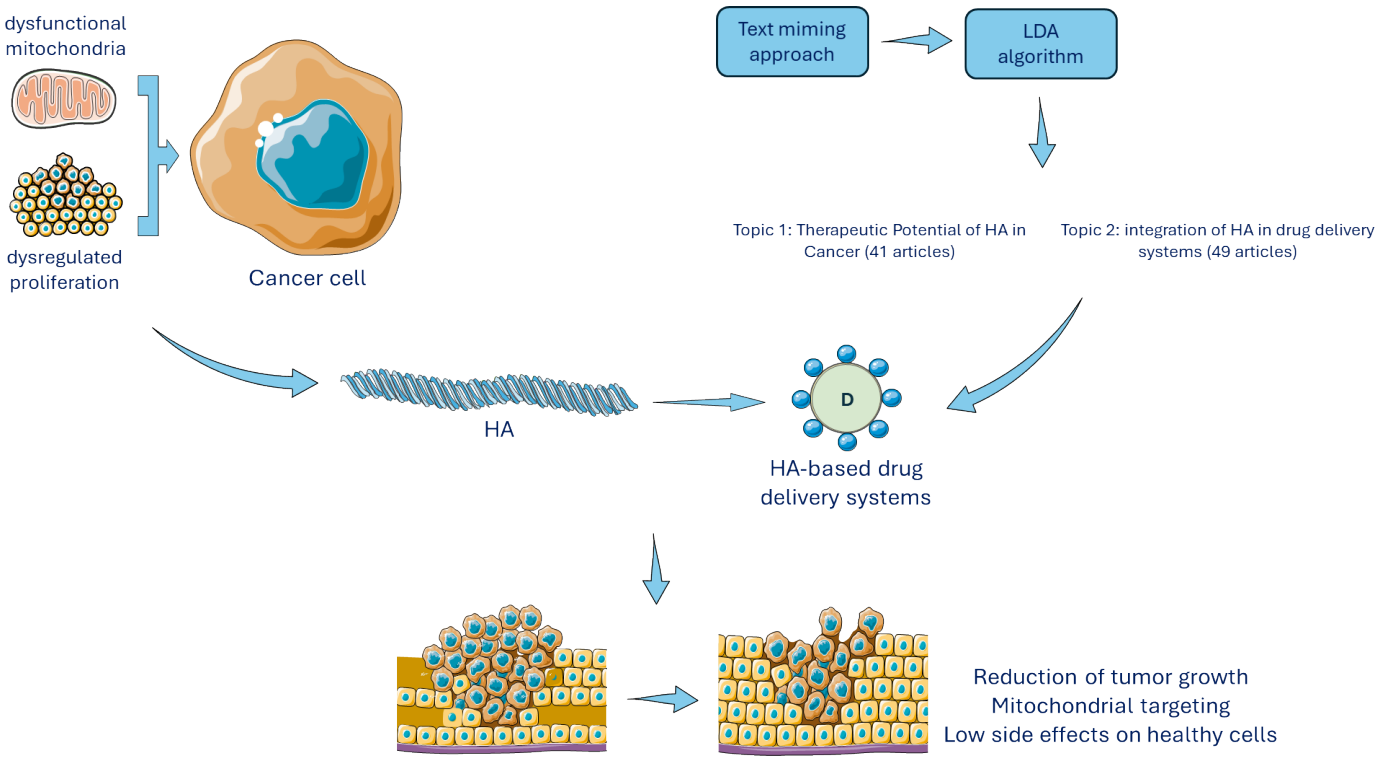
Abstract: Background: Mitochondrial alterations play a crucial role in the development and progression of cancer. Dysfunctional mitochondria contribute to the acquisition of key hallmarks of cancer, including sustained proliferative signalling, evasion of growth suppressors, and resistance to cell death. Consequently, targeting mitochondrial dysfunction has emerged as a promising therapeutic strategy. Hyaluronic acid (HA), a naturally occurring glycosaminoglycan, has garnered significant attention due to its multifaceted roles in cancer biology.
Methods: We employed a Systematic Literature Review (SLR) approach to examine a collection of 90 scientific publications using a text mining technique leveraging the Latent Dirichlet Allocation (LDA) algorithm.
Results: The result of this activity, performed through the MySLR digital platform, allowed us to identify a set of two distinct topics representing the research domain. Specifically, Topic 1 comprised 41 papers, while Topic 2 comprised 49 papers.
Conclusions: The computational analysis highlighted that the integration of HA into drug delivery systems represents a promising approach to enhance the effectiveness and safety of cancer therapies. The discussed clinical trials provided compelling evidence of the potential of HA-based treatments in targeting cancer cells while minimizing adverse effects on healthy tissues.



Graphical Abstract. The image shows the therapeutic potential of HA-based drug delivery systems targeting cancer cells. Using a text mining approach and LDA algorithm, two key topics are identified: HA's therapeutic potential in cancer and its role in drug delivery. HA is used to deliver drugs directly to cancer cells, leading to tumor growth reduction, mitochondrial targeting, and minimal side effects on healthy cells.

Keywords: hyaluronic acid, hyaluronan, cancer, tumor, mitochondria, machine learning, clinical trials, apoptosis

1. Introduction

Cancer continues to pose a significant global health challenge, demanding the need for innovative therapeutic approaches. Despite systemic chemotherapy effectively targeting cancer cells, it can also harm healthy cells, leading to adverse effects. Additionally, chemotherapy drugs exhibit low biostability and are quickly cleared from the bloodstream, further emphasizing the need for targeted delivery systems to reduce off-target effects and improve in vivo stability [1]. Mitochondria, which are integral to cellular metabolism and signaling pathways, have emerged as essential regulators in cancer initiation, progression, and resistance to therapy [1]. Given their central role, targeting mitochondria with therapeutic agents holds significant promise for disrupting cancer cell viability and overcoming treatment resistance. However, achieving precise mitochondrial targeting while minimizing negative impacts on healthy tissues remains a considerable challenge [2]. Drug delivery systems, particularly those utilizing biocompatible polymers, offer a promising avenue for enhancing the specificity and efficacy of anticancer therapies [3]. In the field of nanomedicine, various ligands, such as small molecules, antibodies, and peptides, have been used to decorate nanosystems carrying therapeutic agents and to target specific receptors on target cells [4-7].

Among these ligands, the extracellular glycosaminoglycan HA has been extensively studied and employed to create nanocarriers that specifically target the integral membrane receptor protein CD44, expressed at elevated levels in multiple types of cancer cells [1].

Upon binding to CD44 at the cell surface, HA initiates a cascade of cellular events, including growth, differentiation, and migration. It also plays a significant role in scavenging oxygen radicals, and its binding to the CD44 receptor appears to mediate most of its endogenous effects [8]. The CD44 receptor has been found to be crucial for the normal function and survival of various cell types. For example, it can promote resistance to apoptosis in colonic epithelium through a mitochondria-controlled pathway [9], and it can

prevent stress-induced, p53-dependent cytostatic and apoptotic signals in non-transformed cells [10]. CD44 also plays a critical role in supporting tumor growth and metastasis in experimental models of solid cancers [11].

The interactions of HA with CD44, and of CD44 with the actin cytoskeleton and multiple growth factor receptors, are critical controllers of cell-microenvironment communication and play an important role in cancer initiation and progression [12, 13]. High molecular weight HA (HMW-HA), characteristic of the homeostatic environment, clusters CD44 receptors and supports stable interaction with ERM proteins and the actin cytoskeleton. In contrast, low molecular weight HA (LMW-HA), created under pathological conditions by the action of hyaluronidases and/or reactive oxygen and nitrogen species, can de-cluster CD44 and leave it more susceptible to proteolytic cleavage. Cleavage results in shedding of the CD44 extracellular domain, and is followed by additional proteolytic cleavage within the CD44 transmembrane domain and liberation of the intracellular tail to the cytoplasm. The CD44 tail is translocated to the nucleus where it interacts with transcription factors controlling expression of multiple genes, notably matrix metalloproteinase -9 (MMP-9). An alternative fate occurs for CD44 carrying modifications such as S-acylation, sialylation, and/or phosphorylation, driving its localization into lipid rafts, where HA and CD44 can be endocytosed together. Both the shedding and endocytosis fates of CD44 contribute to processes such as changes in cell-cell adhesion and cell migration, critical to the initiation, growth and metastasis of tumors. Internalized HA may also protect DNA from oxidants [14].

There is evidence for a connection between HA, CD44, and mitochondrial function, although the mechanism has not been elucidated. HA modulates mitochondrial roles in cellular metabolism, energy production, and apoptotic signaling processes that are dysregulated in cancer [15]. Recent studies have highlighted the protective effects of HA on mitochondria and its genetic material in the presence of oxidative stress, which can be detrimental to mitochondrial function [16]. HA has demonstrated its ability to preserve the energy production, which is one of the essential functions of mitochondria. Furthermore, HA has shown mitoprotective and genoprotective properties, safeguarding the mitochondria from damage. Additionally, HA has been found to enhance cell survival, particularly in cells that have experienced damage from reactive oxygen species (ROS) and reactive nitrogen species [15]. Several studies have identified that HMW-HA functions as an antioxidant, reducing the damage caused by ROS [17]. HMW-HA treatment has also been associated with increased cell viability by reducing the number of apoptotic cells [18]. HMW-HA has been found to inhibit the cleavage of procaspase 9 and the release of cytochrome c from mitochondria [18]. In contrast, LMW-HA can have opposing effects. Tumor tissues exhibit enhanced formation of ROS as a consequence of higher metabolic activity, increased activity of NADPH oxidase, or mitochondrial malfunction. The breakdown of HA in the tumor environment, caused by increased hyaluronidase (HYAL) expression and higher ROS accumulation, has been linked to the invasion of neoplastic cells [19, 20]. The chemical changes of HA caused by ROS may negatively impact the formation, structure, and repair of the extracellular matrix (ECM) [21]. The HA-rich ECM contributes to the recruitment of mesenchymal stem cells, which are the source of cancer-associated fibroblasts (CAFs), supporting tumorigenesis and tumor angiogenesis [22].

While the use of HA in nanomedicine has been demonstrated to be widely advantageous due to its biocompatibility and biodegradability, making it safe for clinical applications [23, 24], recent findings also suggest that HA could play a vital role in protecting mitochondria and enhancing their function in cancer cells. However, the exact relationship between HA, CD44, and mitochondrial regulation remains underexplored and warrants further research.

In this review, we aim to investigate the role of HA in drug delivery systems, particularly in the context of cancer therapy. We discuss the mechanisms by which HA targets CD44 receptors on cancer cells and enhances drug delivery to mitochondria, which are critical for improving therapeutic outcomes. This review also presents a novel perspective by integrating insights derived from machine learning approaches, examining patterns in scientific literature over the past decade. By applying machine learning, we aim to uncover trends that may be overlooked by conventional methods, particularly in the context of HA's role in drug delivery systems targeting mitochondrial alterations in cancer. Our objective is to deepen the understanding of HA's potential in facilitating

effective drug delivery to mitochondria, while identifying key areas that require further investigation to develop targeted therapeutic strategies.

Furthermore, this review emphasizes the advantages of using HA in nanomedicine, given its biocompatibility and biodegradability, which make it a safe and viable option for clinical applications. We also highlight the promising findings from clinical trials and call for more research to address existing knowledge gaps, particularly in optimizing HA-based delivery systems and understanding the mechanisms of resistance. Together, these insights point to the strong potential of HA in cancer therapy, with its diverse roles in tumor progression and therapeutic interventions.

2. Materials and Methods

In this study, a machine learning approach was employed to systematically analyze a large collection of scientific literature, aiming to extract critical knowledge aligned with the study's objectives. Unlike traditional algorithms designed to handle structured and numerical data, scientific literature consists mainly of unstructured textual documents, such as research papers. To manage this unstructured data, the LDA algorithm was selected for its effectiveness in topic modeling and information extraction from such texts [25]. The LDA algorithm was applied to process and interpret the textual data contained within the research papers. LDA is a generative probabilistic model that identifies latent topics in a corpus of documents by examining the distribution of words across the texts. In this context, the algorithm was used to uncover patterns and extract relevant topics from the scientific literature, which aligned with the focus of the study.

The platform employed for this analysis was MySLR, which integrates LDA to enhance its ability to handle large datasets of unstructured text. MySLR replicates human-like intelligence in its processing capabilities, enabling it to sift through extensive volumes of research articles, analyze content, and identify relationships between topics across different papers. This automated system reduces manual effort and improves the consistency and accuracy of literature reviews by efficiently identifying key themes and concepts within the body of research [26]. The platform is accessible at https://myslr.unical.it upon registration.

The methodological approach is structured around three distinct steps [27]:

- Paper Location and Selection: Relevant research articles were identified based on predefined criteria. The platform's algorithm automatically filtered out irrelevant publications, ensuring alignment with the study's goals.
- Paper Analysis: The selected articles were analyzed using the LDA algorithm, which identified underlying topics by examining word distributions within the texts, revealing central themes and patterns.
- Results Presentation: The LDA analysis results were presented as topic distributions across the papers, highlighting key themes and illustrating relationships between different studies.

This approach provided a comprehensive understanding of the literature and uncovered important insights for future research.

2.1 Paper location and selection

We undertook an extensive search across PubMed, Scopus, and Web of Science (WOS) databases until March 15, 2024 aiming to identify relevant studies in the last 10 years.

Our search targeted research on the therapeutic potential of HA in modulating mitochondrial alterations in cancer using the search terms: (“hyaluronic acid” OR “hyaluronan” OR “hyaluronate”) AND (“tumor” OR “cancer”) AND (“mitochondria”). Following the search process, a total of 322 papers were identified (139 from Scopus, 100 from WOS, and 83 from PubMed).

2.2 Paper analysis

We employed a text-mining approach on the final set of ninety papers to elucidate the primary research themes concerning the potential of HA in modulating mitochondrial alterations in cancer. This method exploited LDA, a statistical technique that assigns topics to each document based on word frequency and occurrence. In the domain of Natural Language Processing, LDA serves as

a statistical framework to identify abstract "topics" or themes within a collection of documents, autonomously discerning them based on word patterns.

The LDA algorithm identified two overarching topics, associating them with the keywords extracted from the texts. Each document was accurately assigned its respective semantic topic, generating outputs consisting of sets of relevant keywords representing each topic and a document-term matrix illustrating the statistical relationship between papers and topics.

Following the recommendations of Blei et al. [25], we selected the number of topics (k) as two, resulting in satisfactory topic coherence (-1.11) ensuring ease of interpretation for human readers. Topic coherence evaluates the semantic consistency of highly scored words within a topic, thereby assessing the quality of topic modeling. Subsequently, after removing duplicates, we examined a total of 146 papers, applying inclusion and exclusion criteria to identify eligible studies.

The analysis of pertaining papers revealed limitations in artificial intelligence, as 36 papers selected by the platform did not align with the specified keywords.

Hence, pertaining papers meeting the criteria were independently evaluated based on titles, abstracts, and full texts, resulting in 90 journal articles included in the final set of eligible documents for further analysis. So, we applied text mining to the final set of 90 papers. This process, based on LDA, allocated each document a distribution across a certain number of topics, treating documents as probability distributions of topics and topics as distributions of words. The algorithm identified three main topics related to the keywords extracted by the LDA procedure, accurately assigning each text its corresponding semantic topic. The output included sets of relevant keywords representing each topic and a document-term matrix illustrating the statistical relationship between papers and topics.

2.3 Results presentation

In the "Results and Discussion" sections, we elaborate on the final stage of our methodological approach, which involves providing a comprehensive description and discussing the outcomes obtained from the LDA procedure. This entails a meticulous human-based review of significant papers clustered around the two identified topics by MySLR.

Following this stage, MySLR offers a dropdown menu enabling the identification of studies classified as articles, reviews, articles in early access, and notes. Notably, we did not encounter editorial articles, meta-analyses, letters to the editors, short communications, errata, book chapters, notes, opinions, personal comments, or retracted publications during our analysis.

The selection algorithm is visualized in the flowchart presented in Figure 1, outlining the steps taken to identify and classify relevant studies within our methodology.

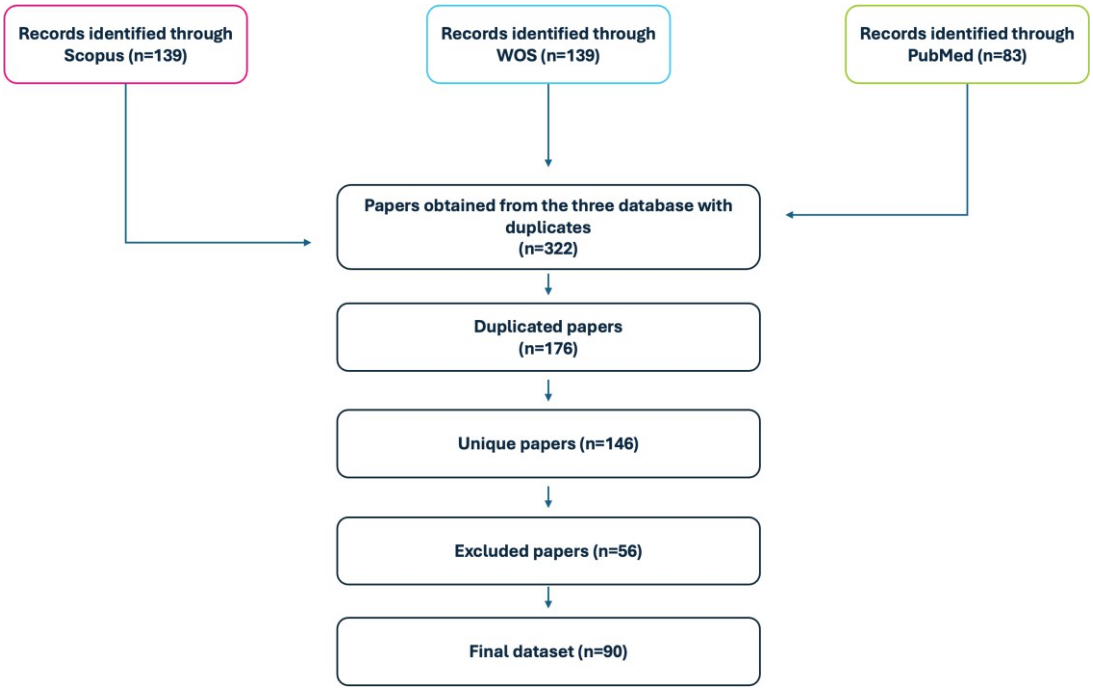


Figure 1. Flow chart of the analyzed papers. This flow chart illustrates the systematic process followed for identifying and selecting relevant research articles for this study. The literature search encompassed three databases: Scopus, WOS, and PubMed, resulting in an initial collection of 322 records. After removing 176 duplicate entries, 146 unique papers were assessed. Following a thorough screening based on predefined inclusion and exclusion criteria, 56 papers were excluded due to irrelevance or insufficient data, leading to a final dataset of 90 papers for analysis. This methodical approach ensures the reliability and relevance of the studies included in the analysis, thus strengthening the findings of this research.

3. Results

The initial literature search, conducted using the MySLR platform, identified a total of 146 unique studies. From this pool, we excluded irrelevant papers, such as reviews and notes. This process also highlighted some limitations of artificial intelligence, as 36 papers selected did not meet the specified criteria, emphasizing the importance of human oversight.

Ensuring the reproducibility of the results obtained through this approach is crucial for maintaining the robustness of our conclusions. After a preliminary evaluation of topic coherence, we set the number of topics (k) to two, resulting in satisfactory topic coherence (-1.11) ensuring ease of interpretation for human readers.

Hence, we ran the LDA algorithm multiple times (5) on the same dataset (with the same parameters) to obtain different sets of topics due to the probabilistic nature of the algorithm. We evaluated the coherence scores of these topics across different runs to assess how stable and consistent the topics are. The coherence coefficients obtained from the five algorithm runs were highly consistent. The variance (0.00115) and standard deviation (0.03391165) indicate minimal dispersion between the coherence scores, demonstrating that the runs produced stable results (Fig. 2).

This level of transparency enables other researchers to replicate our study and verify the results, contributing to the overall credibility of the research.

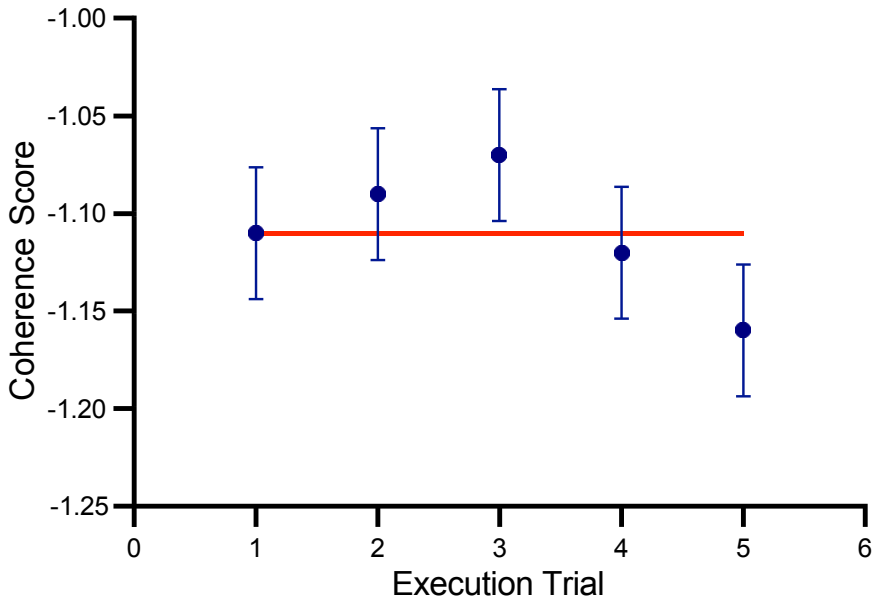


Figure 2. Scatter plot representing coherence scores across different executions. The blue dots indicate the coherence scores for each trial, and the error bars represent the standard deviation. The red line shows the average value of the scores. As shown, the points are closely clustered, highlighting the low variability of the results across trials.

In this way 90 studies were analyzed. Utilizing the LDA procedure, relevant keywords associated with the identified topics were extracted.

In particular, topic 1 comprises 41 papers, while Topic 2 includes 49 papers. Through keyword analysis, the central theme of each topic was discerned based on their respective weights. The weight of each keyword is determined by its frequency of occurrence within the topic, considering word associations. The data generated by the MySLR platform, depict the final weight of the two topics, considering the weight of each keyword, the frequency of keyword repetition, and keyword associations. Documents related to Topic 1 have a cumulative weight of 41,655, whereas those associated with Topic 2 have a cumulative weight of 49,345.

It has been observed that for Topic 1, the most relevant keywords are 'mitochondrial targeting,' 'apoptosis,' and 'CD44,' while for Topic 2, we found 'nanocarrier,' 'liposomes,' and 'drug loading'.

From the MySLR platform it is possible to obtain data about the number of articles published per year, depicted in Figure 3. It is noticeable that there was a significant increase in publications from 2019 forward.

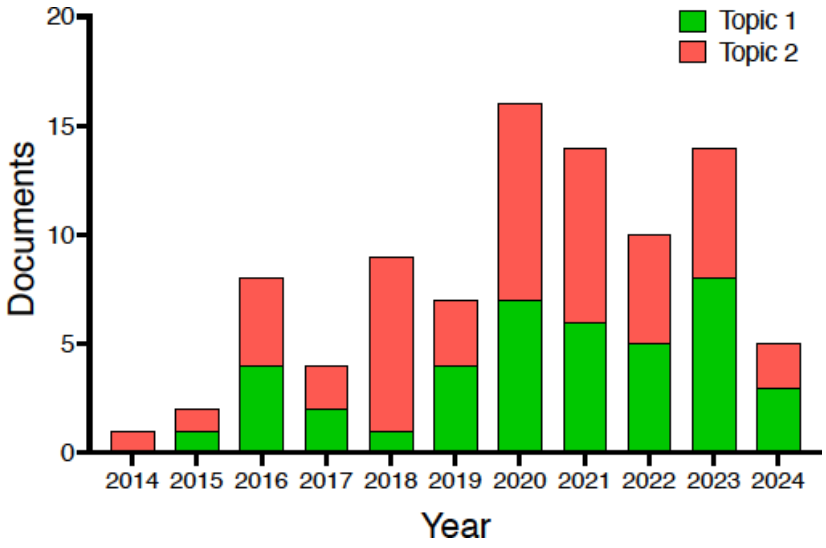


Figure 3. Papers and topics publications trends over the time. This bar chart illustrates the annual publication trends of research papers categorized into two distinct topics from 2014 to 2024, as extracted from the MySLR platform. The green bars represent documents associated with Topic 1, while the red bars denote those related to Topic 2. The data indicate a significant increase in publications, particularly from 2019 onward, highlighting the growing interest and research activity in these topics over the years. This trend underscores the evolving landscape of research focused on the targeted applications of HA in cancer therapies, reflecting the expanding body of knowledge and ongoing investigations in this critical area of study.

Machine learning also enables us to visualize the trends of research papers and related topics over time. As shown in Figure 3, there has been a significant increase in interest in this research area since 2019. Additionally, while the overall research topic has attracted growing attention from researchers in recent years, the trends for the two identified subtopics differ. Specifically, Topic 1 has seen a rapid increase since 2020, with a notable peak in interest in 2023 (Fig. 3). Conversely, Topic 2 grew steadily over time, reaching its highest peak in 2020 (Fig. 3). These differing trends highlight the dynamic nature of research interests and the evolving focus within the field.

3.1 Topic 1: Hyaluronic Acid in Targeted Cancer Therapies and Nanotechnology

Upon examining the top 30 most relevant terms and their frequencies within papers grouped under the first topic (Fig. 4A), and analyzing the 41 papers clustered around this topic, it became evident that the primary focus of this initial topic was centered on the development of targeted therapies for cancer. Specifically, there was a notable focus on approaches involving mitochondria and apoptosis pathways. The utilization of nanoparticles (NPs) in conjunction with specific ligands such as HA and the CD44 receptor, emerged as crucial for enhancing drug delivery efficiency and cancer cell targeting in different tumour types (Table 1). NPs are drug delivery systems that allow controlled and regulated drug release, accessible handling, exceptional availability and biocompatibility, good stability, and increased drug solubility in vivo; in addition, their geometry and nanoscale size greatly improve drug loading rate, release efficiency, long-term circulation time, drug pharmacokinetics, and pharmacodynamics (Table 1).
Moreover, the discussion highlighted the significance of reactive oxygen species, calcium overload, and oxidative stress in both cancer progression and therapeutic interventions. Additionally, the identified keywords pointed towards a keen interest in comprehending and targeting cancer stem cells, along with exploring immunogenic cell death as a potential therapeutic avenue.
Overall, the overarching themes underscored a comprehensive exploration of metabolic processes and their implications in diseases like cancer, alongside investigations into nanoscale drug delivery systems and the complexities of the tumor microenvironment and associated cellular responses.

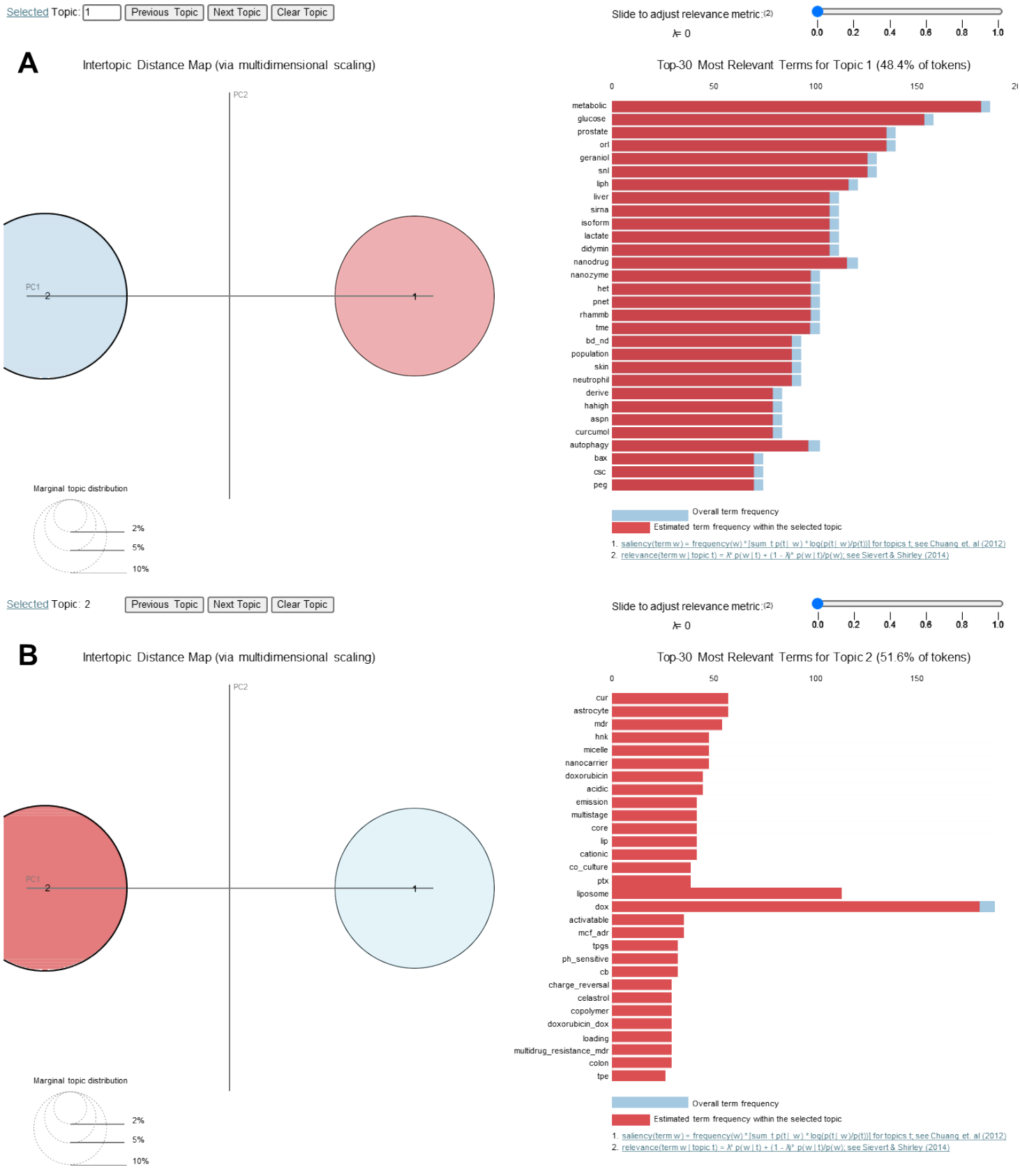


Figure 4. The Inter-topic Distance Map illustrates the relationship between Topic 1 (A) and Topic 2 (B). This figure illustrates the inter-topic relationships derived from the LDA analysis using the MySLR platform. Panel A displays the Inter-topic Distance Map for Topic 1, represented by Circle 1, while Panel B depicts Topic 2, represented by Circle 2. The positioning of each circle indicates the proximity of the two topics in the multidimensional space, suggesting their thematic similarities and differences. Adjacent to each topic's map is a bar chart showcasing the top 30 most relevant terms associated with that topic, indicating the

frequency of terms used within the respective discussions. The relevance metrics reflect the strength of associations between the terms and their respective topics, with Topic 1 covering 48.4% of tokens and Topic 2 covering 51.6%. This visualization provides valuable insights into the underlying themes and keywords that define the discourse within the scientific literature related to these topics.

Table 1. The Targeting Strategies and Biological Action of HA in Cancer Therapy.

Patological conditions	**HA action**	**Adopted strategy**	**Advantage**	**Refs.**
Lung cancer	Targeting of CD44 receptor	Hyaluronidases degrade exogenous- HA in the tumor matrix and tumor cell lysosomes aiding mitochondrial targeting	HA has been shown to halt tumor spreading	[28-33]
Liver cancer	Targeting of CD44 receptor	Exogenous-HA serves as nano-carrier material and show high drugs loading capacity	HA achieves excellent biocompatibility	[34]
Breast cancer	Targeting of CD44 receptor	Exogenous-HA mediates the endocytosis and the accumulation of the antitumoral drug in the tumor site Hyaluronidases degrade endogenous-HA in the tumor matrix and tumor cell lysosomes aiding mitochondrial targeting	HA facilitates tumor recognition and improves drug circulation and stability	[35-47]
Gastrointestinal cancer	Targeting of CD44 receptors	Exogenous-HA mediates the endocytosis and the accumulation of the antitumoral drug in the tumor site	HA facilitates tumor recognition	[48]
Prostate cancer	Targeting of CD44 receptors	Exogenous-HA mediates the endocytosis and the accumulation of the antitumoral drug in the tumor site	HA serves as a carrier to enhance drug effectiveness	[49, 50]
Lung cancer	Targeting of CD44 receptors	Exogenous-HA enhances drug accumulation in tumoral cells	Mitochondrial damage induced by the production of ROS	[51, 52]
Esophageal carcinoma	Inhibition of HA synthesis	The inhibition of endogenous-HA synthesis synergistically enhanced the effectiveness of antitumoral drug treatment	Enhanced efficacy of anticancer therapy	[53]

Cancer associated fibroblasts	Inhibition of HA synthesis	The inhibition of endogenous-HA synthesis synergistically enhanced the effectiveness of antitumoral drug treatment	Enhanced efficacy of anticancer therapy	[54]
Hepatocarcinoma	Targeting of CD44 receptors	Exogenous-HA mediates the endocytosis and the accumulation of Mn/HA-carbon dots (Mn/HA-CD) in the tumor site	Mn/HA-CDs in mitochondria inhibits superoxide dismutase, amplifying ROS damage, enhancing photodynamic therapy	[55]
Amelanotic melanoma	Targeting of CD44 receptors	Exogenous-HA mediates the endocytosis of antitumoral drugs, aiding mitochondrial targeting and enhancing hypoxia in the tumor microenvironment	Inhibition of cancer progression	[56]
Urothelial carcinoma	Targeting of CD44 receptor	Exogenous-HA contributes to the aggregation of heteronemin (HET) onto polymeric NPs, enhancing the activity of HET against tumor cells	Incorporation of HA in the NPs formulation helps to minimize the toxicity of HET on normal cells, enhancing the safety profile of the treatment	[57]
Cervical cancer	Inhibition of HA synthesis	The inhibition of endogenous-HA synthesis synergistically enhanced the effectiveness of antitumoral drug treatment	Enhanced efficacy of anticancer therapy	[58]
Leukemia	Inhibition of HA synthesis	The inhibition of endogenous-HA synthesis synergistically enhanced the effectiveness of antitumoral drug treatment	Enhanced efficacy of anticancer therapy	[59]

In Table 1, the use of HA in cancer therapy has been explored, highlighting its crucial role and interaction with CD44 receptors, as well as the inhibition strategies of HA synthesis. This underscores the superior efficacy of drug-loaded system conjugated with HA over traditional system in combating tumor progression. HA-coated NPs delivery systems have shown enhanced drug accumulation in CD44-positive tumor cells, improving antitumor efficiency through mitochondrial damage induced by ROS. These NPs act through various mechanisms, such as targeting mitochondria-related pathways, inducing mitochondrial stress, and facilitating apoptosis in cancer cells. Further studies on supramolecular nanoassemblies and mitochondria-targeting nanoplatforms indicate high therapeutic efficacy against malignant tumors, with controlled aggregation and enhanced ROS production significantly improving photodynamic therapy (PDT) and reducing metastasis-related mortality [51, 52, 55, 56]. Moreover, they offer targeted drug delivery, improved drug accumulation in tumor cells, and reduced systemic toxicity compared to traditional chemotherapeutic agents. Additionally, they show promising results in overcoming multidrug resistance (MDR), inhibiting tumor growth, and suppressing metastasis, making them a valuable approach for cancer therapy [41]. For instance, in lung cancer, exogenous-HA targeting CD44 receptors has been shown to inhibit tumor progression by facilitating mitochondrial targeting through exogenous HA degradation

in the tumor matrix and tumor cell lysosomes by hyaluronidases [18, 28, 31, 45]. For instance, Sinulariolide (SNL), extracted from Sinularia flexibilis, demonstrates significant anticancer activity when conjugated with HA-NPs to form HA/SNL/NPs aggregates. These aggregates are more effective at inducing apoptosis in A549 lung cancer cells at lower SNL doses compared to pure SNL, primarily via mitochondria-related pathways [28]. Moreover, a novel type of organic–inorganic hybrid supramolecular nanofiber composed of gold nanorods, mitochondrion-targeting-peptide-coated iron oxide nanoparticles, and HA-modified β-cyclodextrin has shown promise in cancer treatment. These photothermal nanofibers induce severe mitochondrial damage in human adenocarcinoma cells (A549) and significantly suppress metastasis in tumor-bearing mice upon near-infrared laser irradiation. The combination of HA receptor targeting, mitochondrial targeting, and high photothermal conversion efficiency makes these nanofibers a potent nanotherapy for cancer and metastasis-related malignancies [31]. Additionally, inducing immunogenic cell death (ICD) through mitochondrial stress represents a robust therapeutic strategy. The development of a dendrimer-based nanoplatform, DIH, which combines mitochondria-targeted PDT, mild-temperature photothermal therapy (MTPTT), and ICD-induced immunotherapy, shows potential for effective cancer treatment. The DIH platform targets mitochondria with high precision, producing ROS and mild heat upon near-infrared light irradiation, thereby enhancing ICD and inhibiting tumor growth. This innovative approach provides a promising guideline for designing effective ICD inducers in future cancer therapies [45]. Similarly, in non-small cell lung cancer, exogenous -HA's interaction with CD44 receptors facilitates the specific targeting of miRNA to mitochondria within cancer cells, showcasing its potential as a targeted therapeutic agent [29,30, 33]. In a study involving forty patients with extensive stage small cell lung cancer (SCLC), HA-irinotecan treatment demonstrated notable efficacy and tolerability, with an overall response rate of 50% and a median progression-free survival of 14.4 weeks in the second-line cohort [29]. Furthermore, preclinical studies in SCLC have elucidated the efficacy of HA as a carrier for chemotherapy and its ability to selectively target CD44-expressing tumor cells [30]. Building on these findings, a clinical study investigated the safety and activity of HA-irinotecan in patients with extensive stage SCLC, revealing encouraging overall response rates and a potential survival benefit in patients with CD44s positive tumors [30]. Finally, recent research has explored the use of mitochondria-targeted miRNA delivery systems for inhibiting lung cancer, demonstrating promising efficacy in inhibiting cell viability and inducing apoptosis [33]. Specifically, let-7b-loaded nanoparticles, designed with HA as a targeting ligand for CD44 overexpressed receptors on NSCLC cells, efficiently localized in the mitochondria of A549 cells, leading to decreased expression of genes involved in mitochondrial oxidative function and ultimately inhibiting lung cancer cell proliferation [33]. In liver cancer, exogenous -HA acts as a nano-carrier material, enhancing drug loading and achieving excellent biocompatibility [34]. In detail this study explored the inhibitory effect of hydroxycamptothecin (HCPT) on human liver cancer cells (HepG-2) by using carboxymethyl chitosan (CMC) and HA to modify graphene oxide (GO), creating a drug delivery system with high biocompatibility and efficiency in reducing proliferation and migration of cancer cells [34]. Additionally, in breast cancer, exogenous -HA targets CD44 receptors to mediate drug accumulation in tumors, providing a strategy for targeted drug delivery. Moreover, the use of HA-coated NPs has been shown to induce mitochondrial stress through ROS generation and control drug release [45], directly interfere with critical metabolic processes for tumor cell survival [40], influence gene expression or protein activity involved in metabolism [44] and trigger the apoptosis pathway [36]. Furthermore, it is a promising strategy for the treatment of metastatic breast cancer by overcoming MDR and targeting mitochondria followed by enhanced apoptosis [41]. The authors developed self-assembled nanodrugs with high drug loading (~68%) using a simple nano-precipitation method, combining a berberine derivative (Ber) and doxorubicin (Dox) [41]. These nanodrugs, modified with DSPE-PEG-folic acid (DSPE-PEG-FA) and HA, successfully altered the target location of Dox from the nucleus to mitochondria, thereby inhibiting the proliferation, invasion, and migration of MDA-MB-231 cells by triggering cell apoptosis. In a xenograft mouse model of MDA-MB-231 breast cancer cells, the nanodrugs demonstrated significant efficacy by repairing mitochondrial protein defects, suppressing MMP-2 and -9 activities, and inhibiting tumor cell proliferation and pulmonary metastasis. This study highlights a promising strategy for the treatment of metastatic breast cancer through targeted mitochondrial therapy and enhanced apoptosis [41]. Finally, it is noteworthy

how the formation of polymeric aggregates of HA/HET (a marine bioactive compound with antitumor activities) demonstrates a stronger capacity for inducing apoptosis through mitochondrial depolarization, as demonstrated in Urothelial carcinoma [57]. These findings underscore the potential of HA targeting in cancer treatment across various pathological conditions, presenting new avenues for therapeutic development and personalized medicine.

3.2 Topic 2: Type of Drug Delivery Systems Based on Hyaluronic Acid for Cancer Therapy

The examination of the top 30 most relevant terms and their frequency within papers grouped around the second topic (Fig. 4B) suggests a focus on developing targeted therapies for cancer by exploiting mitochondrial targets, utilizing HA-based drug delivery systems, and addressing MDR challenges. Nanotechnology, supramolecular chemistry, and imaging techniques (are explored as potential approaches to enhance drug delivery and treatment efficacy. In particular, this second topic focuses on various aspects of drug delivery systems, including subcellular targeting, circulation stabilization, nanoparticle surface modification, and drug metabolism manipulation. Targeting strategies encompass targeted drug accumulation in tumor cells, sensitivity to the tumor microenvironment, CD44 receptor targeting with functionalized HA, and selective delivery of therapeutic agents to tumor cells and mitochondria. Additionally, the importance of mitochondrial targeting for amplifying therapeutic effects and overcoming MDR is highlighted (Table 2). Studies clustered in this topic are listed in table 2.

Table 2. Targeted NPs Strategies for Cancer Therapy.

Categorie NPs	Targeted Strategies	refs
NPs for subcellular targeting and circulation stabilization	MDR and improving drug accumulation in tumor cells	[60-65]
	Maximizing therapeutic efficacy through targeting strategies	[60-65]
	Sensitivity to the tumor microenvironment	[63, 64]
	NPs surface modification	[61-65]
	Targeted Drug Delivery and Metabolism Manipulation	[65, 66]
NPs for CD44 receptor targeting	CD44 Receptor Targeting Strategies with HA Functionalized NPs	[67-71]
	Selective Delivery of Therapeutic Agents to Tumor Cells and Mitochondria	[69, 72-75]
NPs for combined therapy and overcoming MDR	NPs for combined therapy and overcoming MDR	[76-81]
	Mitocondrial targeting for therapeutic effects amplification	[76, 77, 80-83]
NPs for photodynamic therapy and magnetic resonance	Mitochondria-targeted therapies	[84-86]
	Hybrid nanoplatforms for combined therapy	[83, 87-92]

NPs sensitive to pH and redox for subcellular targeting	[93, 94]

The Table 2 provides an overview of the targeted strategies employed in the use of NPs for cancer therapy. A wide range of approaches is highlighted, including subcellular targeting, circulation stabilization, NPs surface modification, and drug metabolism manipulation. Targeting strategies encompass targeted drug accumulation in tumor cells, sensitivity to the tumor microenvironment, CD44 receptor targeting with functionalized HA, and selective delivery of therapeutic agents to tumor cells and mitochondria. Additionally, the importance of mitochondrial targeting for amplifying therapeutic effects and overcoming MDR is highlighted.

In “NPs for Subcellular Targeting and Circulation Stabilization”, the focus lies on targeted drug delivery and anti-tumor treatments, addressing MDR and enhancing drug accumulation in tumor cells. NPs surfaces are tailored to improve targeting and stability, facilitating accumulation in tumor areas. Furthermore, studies by Xiao, S. (2023) and Yu, X. (2021) highlight diverse approaches in cancer therapy, including manipulating cellular metabolism and targeted drug delivery [65, 66]. A critical aspect for tumor growth and migration is sufficient energy generation based on the effective transport of nutrients via abundant blood vessels and subsequent oxidative metabolism in mitochondria. Thus, a strategy to cut off this transport pathway and simultaneously disable the "powerhouse" mitochondria is highly desired for tumor treatment. In this context, a bionic nanocarrier with a core-shell-corona structure was developed for selective and effective tumor therapy via stepwise destruction of existing tumor vessels, inhibition of tumor angiogenesis, and dysfunction of tumor mitochondria [65]. The core of this nanocarrier consists of mesoporous silica nanoparticles (MSNs) co-loaded with combretastatin A4 phosphate (CA4P) and vitamin K2 (VK2), responsible for vascular destruction and mitochondrial dysfunction after cargo release. The shell of N-tert-butylacrylamide (TBAM) and tri-sulfated N-acetylglucosamine (TSAG) serves as an artificial affinity reagent against vascular endothelial growth factor (VEGF) for angiogenesis inhibition. To ensure these actions occur only in the tumor, a HA corona was introduced to endow the nanocarrier with tumor-targeting properties and stimulus-responsiveness for accurate therapy. Furthermore, a novel multistage-targeted celastrol delivery system (C-TL/HA) was developed to overcome the clinical challenges of celastrol. This system utilizes cationic liposomes loaded with celastrol and electrostatically bound to HA, improving antitumor efficacy and reducing side effects compared to free celastrol [66]. The HA coating enhances stability and safety in vivo while facilitating tumor targeting through recognition of CD44 receptors on tumor cells. In conclusion, these innovative approaches offer promising prospects for clinical application in tumor therapy. In “NPs for CD44 Receptor Targeting”, HA-functionalized NPs effectively target CD44 receptors, enhancing anti-tumor efficacy. This approach, demonstrated by Liang et al. (2015) and Xie et al. (2023), boosts therapeutic effectiveness [67, 71]. The potent antitumor activities of silver NPs (AgNPs) have attracted great attention. However, the application of AgNP is restricted by its non-specific delivery and poor cellular uptake. To address this, a novel HA-based strategy for the green synthesis of AgNP was developed, where HA serves as both the reducing agent and stabilizer. Importantly, HA, a ligand of CD44, enables HA-modified AgNP to target CD44 receptors overexpressed in many cancer cells, significantly enhancing intracellular delivery via CD44-dependent endocytosis. This modification not only improved antitumor efficacy but also leveraged multiple mechanisms, including mitochondrial membrane potential decline, cell-cycle arrest, apoptosis, and autophagy [67]. Delivering traditional DNA-damaging anticancer drugs into mitochondria is a promising chemotherapy strategy. However, the impermeability of the mitochondrial inner membrane impedes drug delivery. In this context, a prodrug, camptothecin (CPT)-triphenylphosphine (TPP), modified with HA via electrostatic adsorption (HA/CPT-TPP, HCT), was used to mediate mitochondrial accumulation of CPT. These nanoparticles showed enhanced drug accumulation in cancer cells through tumor targeting. HCT entered acidic lysosomes via endosomal transport, where HA was degraded by hyaluronidase, exposing the positively charged CPT-TPP, which then accumulated in the mitochondria. This led to significant disruption of mitochondrial structure and function, increased ROS levels, energy depletion,

and enhanced lung cancer cell apoptosis via caspase-3 and caspase-9 activation. In vivo, HCT administration in nude mice bearing A549 xenograft tumors greatly inhibited tumor growth, showcasing the potential of mitochondria-targeted delivery of CPT as an effective antitumor strategy [72]. Additionally, NPs deliver therapeutic agents to mitochondria, increasing treatment efficacy [68, 95]. Integrating this capability with the development of intrinsically mitochondria-targetable nanosystems represents a significant advancement in subcellular structure-oriented precise cancer therapy. The construction of HA-IR825-Chol nanostructure demonstrates enhanced photostability and desirable photothermal properties, enabling selective accumulation in mitochondria and inducing severe mitochondrial damage upon laser irradiation, leading to cell apoptosis. Furthermore, the incorporation of HCPT into HA-IR825-Chol NPs for combined chemo-photothermal therapy showcases promising results in efficient tumor eradication. Similarly, the DTX-HA nanomedicine, responsive to intracellular stimuli, exhibits efficient cellular uptake and apoptosis induction, demonstrating remarkable therapeutic efficacy both in vitro and in vivo. These approaches highlight the potential of nanomedicine in targeted cancer therapy, offering precise subcellular drug delivery with improved treatment outcomes [68, 73]. Integrating strategies like combining CD44 receptor targeting with mitochondrial targeting enhances anti-tumor therapy [69, 73]. The development of core-shell NPs loaded with mitotropic DOX-TPP+ showcased promising results in breast cancer treatment. These NPs exhibited high encapsulation efficiency and successful induction of apoptosis in vitro, along with significant anticancer activity in Solid Ehrlich carcinoma (SEC)-bearing mice in vivo [69]. Similarly, our study utilizing MSN-DPH demonstrated preferential uptake by cancer cells via CD44 receptor-mediated endocytosis, followed by efficient accumulation in mitochondria due to the mitochondrial-targeting ability of TPP. Furthermore, degradation of HA facilitated the release of Dox specifically within cancer cells, resulting in efficient cancer cell killing while exhibiting lower cytotoxicity to normal cells [73]. These findings underscore the potential of targeted nanosystems in improving the efficacy and safety of cancer therapy.

In “NPs for Combined Therapy and Overcoming MDR”, NPs utilize exogenous -HA as a targeting ligand and incorporate various therapeutic agents to target both tumor cells and mitochondria, achieving enhanced therapeutic effects. Studies by Xiong et al. (2016) and Zhao et al. (2024) demonstrate the efficacy of dual-targeted delivery in overcoming MDR [75, 81]. Dual-targeted nanoparticles represent a promising strategy for enhancing anti-proliferation effects by targeting multiple key sites within tumor cells. The development of HA modified hydroxyapatite (HAP) NPs (HAP-NPs) offers a dual-targeted approach, exploiting both HA's affinity for CD44 receptors overexpressed on tumor cells and HAP's ability to deliver DOX to nuclei and mitochondria simultaneously. DOX-loaded HAP-HA nanoparticles exhibit high drug loading efficiency and uniform particle size, with confirmed targetability to both mitochondria and nuclei. In vivo studies (on mice and rabbits) demonstrate superior anti-tumor efficacy and reduced toxicity compared to other formulations, positioning DOX/HAP-HA as a promising targeted delivery system for effective cancer therapy [76]. Additionally, the development of a multifunctional nano-bomb, integrating calcium peroxide (CaO_2), β-lapachone, and GSH-sensitive Fe-based coordination polymer, demonstrates dual cascade-amplified tumor chemodynamic therapy (CDT). This HA-modified nano-bomb targets breast cancer cells, releasing cargoes in response to the GSH-rich cytoplasm and self-generating sufficient H_2O_2. The resulting cascade amplification of CDT and induction of severe oxidative stress effectively suppress tumor growth both in vitro and in vivo (on mice), highlighting its potential as a novel approach for tumor therapy with activated ferroptosis and self-supplying H_2O_2 [81]. Additionally, NPs responsive to intracellular stimuli and those co-delivering chemotherapeutics and natural compounds show promise in cancer therapy [77, 95]. Addressing the challenge of precisely co-delivering the chemotherapeutic paclitaxel (PTX) and the natural P-glycoprotein (P-gp) inhibitor, quercetin (QU), into cancer cells, particularly in MDR tumors, is crucial for enhancing anticancer effects. To tackle this, hybrid polymeric NPs (PNPs) were developed, integrating redox-sensitive PTX/polyethyleneimine-tocopherol hydrogen succinate-dithioglycolic acid PNPs with pH-sensitive HA-QU conjugates. These hybrid PNPs efficiently internalize into drug-resistant breast cancer cells via the HA/CD44-mediated endocytosis pathway and escape lysosomal degradation through the "proton sponge effect." Upon intracellular stimuli, the nanoplatform disassembles via pH/glutathione dual sensitivity, releasing QU and PTX. PTX induces tumor cell apoptosis by

diffusing into microtubules, while QU enhances PTX retention by down-regulating P-gp expression. Additionally, tocopherol hydrogen succinate and QU disrupt mitochondrial functions, amplifying intracellular chemotherapy in MDR cancers. In vivo studies in MCF-7/ADR tumor-bearing nude mice demonstrate significant tumor growth suppression by these PNPs, highlighting their potential for combination therapy in MDR cancers [78]. Prodrug self-assembled nanomedicines with cleavable moieties sensitive to intracellular stimuli offer promising avenues in cancer therapy. A docetaxel-HA (DTX-HA) conjugate with cleavable peptide, hydrazone bonds, and disulfide sequences, responsive to MMP, weak acidity, and glutathione (GSH), respectively, was synthesized and self-assembled into nanoparticles for cancer chemotherapy. Characterization via various techniques confirmed the efficient release of the prodrug in simulated intracellular conditions. In vitro studies demonstrated efficient cellular uptake and apoptosis induction in cancer cells, while in vivo studies in tumor-bearing nude mice revealed promising therapeutic efficacy with minimal toxicity to organs, highlighting the potential of these nanomedicines for cancer treatment [96].

Research on "NPs for Photodynamic Therapy and Magnetic Resonance" highlights the efficacy and safety of NPs in clinical applications. Tian et al. (2023) developed a dual-locking fluorescent nanoprobe (HA-DMC) for precise imaging of tumor cell mitochondria [85]. Wang et al. (2023) utilized mitochondria-targeted liposomes (HA PTX-loaded cationic liposome, HA/TT LP/PTX) to overcome MDR and induce cellular apoptosis [86]. Cai et al. (2016) proposed a photosensitizer (DTDPP-HA) for tumor targeting and localized activation [84]. Sahoo et al. (2022) integrated dynamic phototherapy with magnetic resonance imaging for effective combined therapy [91].

In the "NPs Sensitive to pH and Redox for Subcellular Targeting" topic, papers focus on developing pH and redox-sensitive NPs for enhanced cytotoxicity against cancer cells, with the study of Qi et al. (2018) targeting mitochondria and CD44 receptors [94], while Qiu et al. (2014) address MDR in tumor cells [93].

Finally (not in table) Yang, F. (2023) combines PDT with a mitochondria-targeted drug delivery system, sensitizing ferroptosis and promoting apoptosis for enhanced antitumor efficacy [97]. Similarly, Chen, S. (2017) presents a versatile nanoplatform for tumor-targeting synergistic photothermal/chemotherapy, utilizing mitochondria-targeting gold nanostar (AuNS) co-encapsulated with anticarcinogen DOX in a HA protective shell, showing notable tumor inhibition [98].

In summary, the exploration across various topics underscores the multifaceted nature of nanoparticle-based therapies in cancer treatment, showcasing innovative approaches to target-specific drug delivery, overcome drug resistance, and enhance therapeutic efficacy.

3.3 Enhanced Antitumor Efficacy with Hyaluronic Acid: In Vivo and In Vitro Studies

In the field of cancer therapy, the systemic administration of chemotherapeutic agents such as PTX, DOX, and cisplatin represents a key treatment strategy [99, 100]. However, these anticancer drugs can affect healthy cells, leading to various side effects. Additionally, they often exhibit poor biostability and can be rapidly cleared from the bloodstream.

To address these challenges, the development of drug delivery systems capable of precisely targeting cancer cells while minimizing off-target effects is imperative. Nanocarrier-based platforms have emerged as a promising strategy in this regard, as they can be tailored to facilitate targeted drug delivery, thereby enhancing the therapeutic effectiveness of anticancer drugs. HA has shown promise as a component of these drug delivery systems, particularly in cancer chemotherapy. Encapsulating chemotherapeutic agents within HA-based nanocarriers aims to overcome issues such as nonspecific targeting and systemic toxicity. HA's efficacy in drug delivery is attributed to its ability to target specific receptors, notably CD44, which are often overexpressed in cancer cells. This targeted approach enhances drug delivery to cancer cells while minimizing off-target effects, thereby improving the safety and efficacy of cancer treatment [101]. In this paragraph, we evaluated whether there is an advantage in the use of HA-coated-NPs compared to non-coated-NPs in cancer therapy with nanoparticles directed specifically against the mitochondria or mitochondrial function. For this purpose, from the 90 studies used in this work, we extracted only those that presented quantitative comparative

data between HA-coated-NPs and non-coated-NPs in cancer therapy. 13 articles were included in the final analysis, the information of which is listed in Table 3. These studies encompass different types of HA-NPs, such as Sinulariolide-conjugated HA-NPs, HA-PEG modified barberine derivatives, cerium oxide HA-NPs, and others, showcasing their potential to significantly improve therapeutic outcomes in cancer treatment through various mechanisms like increased apoptosis induction, reduced cell migration, enhanced drug delivery, and better stability. The evidence from in vitro and in vivo experiments underscores the promise of HA-coated NPs as a more effective and safer approach in oncology.

Table 3. Comparative Efficacy of HA-NPs versus Non-Coated NPs in Cancer Treatment

Drugs NP platform	**Types of study**	**Parameters**	**HA-NPs**	**Naked NPs**	**Refs.**
Sinulariolide-conjugated HA NPs	In vitro on A549 cells	Apoptosis induction level	50.1%	12.8%	[28]
		Cell cycle arrest (S-G1 phase)	9.2%	3.5%	
		Caspases expression	Higher	lower	
		Side effects	Less	More	
HA-PEG BD	In vitro on A549 cells In vivo on BALB/C-nude mice bearing A549 cells	NPs size	150 nm	125 nm	[32]
		Drug release (after 25 h)	20%	30%	
		Cancer cells survival (5 μg/ml)	40%	60%	
		Mitochondrial membrane potential	50%	30%	
		Apoptosis induction level	50.3%	39.7%	
		Mitochondrial targeting	+10%		
		Caspase 9 activity	1.5	1.2	
		Caspase 3 activity	1.6	1.4	
		Expression of pro-apoptotic proteins (Bax)	Higher	Lower	
		Expression of anti-apoptotic proteins (Bcl-2)	Lower	Higher	
		Tumor growth slowdown	-50%		

CePEI HA-NPs	In vitro on MDA-MB-231	Cytotoxicity in cancer cells	80%	30%	[37]
		Cellular uptake	5 times higher		
HA-camptothecin-NPs	In vitro on MDA-MB-231 MCF-7 HepG2 cells	Cellular uptake (MCF-7)	2 times higher		[38]
		Cellular uptake (HepG2)	8 times higher		
		Cellular uptake (MDA-MB-231)	14 times higher		
		Cytotoxicity (MCF-7)	+12%		
		Cytotoxicity (HepG2)	+20%		
		Cytotoxicity (MDA-MB-231)	+20%		
		Cytotoxicity (normal cells)	-50%		
		Cancer cell migration	-10%		
		Bax expression	1.5 times higher		
		P53 expression	2.6 times higher		
		Bcl-2 expression	-70%		
TPP-DOX-HA-PBPE	In vitro on MCF-7 cells	Accumulation of antitumor drug at tumor site	higher	lower	[40]
		Cytotoxicity in cancer cells	7.3 times higher		
HA-FA-BD-NPs	In vitro on MDA-MB-231 Cells and in vitro on MDA-MB-231 tumor bearing female BALB/c nude mice	Cellular uptake	+24%		[41]
		Apoptosis	+10%		
		Cytotoxicity in cancer cells	+40%		
		Tumor volume reduction	600 mm^3	1200 mm^3	
GNSs-dPG-3BP-TPP-HA	In vitro on MDA-MB-231 Cells and in vitro on Female	Cytotoxicity in cancer cells (20 µg/ml)	50%	35%	[43]
		PTT (temperature)	55°C	48.9°C	

	Balb/c nude mice	OCT4 expression	30%	40%	
		NANOG expression	15%	40%	
		SOX2 expression	25%	40%	
HA-ss-Geraniol NPs	In vitro on PC-3 cells and in vivo on male nude mice	Cytotoxicity in cancer cells	Higher	Lower	[50]
		Cell cycle arrest	21.97%	9.00%	
		Apoptosis induction	8 folds increased		
5-Fu-HA-CS NPs	In vitro on A549 cells	IC_{50} value	8.0 µg/ml	13.1 µg/ml	
		Toxicity in cancer cells (5 µg/ml)	22%	43%	[51]
		ROS production	Increased		
		Expression of anti-apoptotic proteins (Bcl-2)	Lower	Higher	
		Expression of pro-apoptotic proteins (caspases 9, 3 and cytochrome c)	Higher	lower	
MitP-MNP□HACD NPs	In vitro on A546 cells, in vivo on A549-Luc2-tdT-2–bearing nude mice	Cytochrome C expression	0.6	0.9	[52]
		Caspase 3 expression	0.5	0.7	
		Intracellular ATP production	0.2 µg/mg protein	0.28 µg/mg protein	
		Apoptosis induction	0%	25.11%	
HA-HET NPs	In vitro on T24 cells	Cancer cells cytotoxicity	35%	20%	[57]
		Vitality of health cells	90%	60%	
		Number of migratory cells	10%	60%	
		Expression of pro-apoptotic proteins	Higher	Lower	
		Early apoptosis induction	25%	12%	
		Rate of apoptosis	35%	12%	

		Loss of mitochondrial potential	Higher	Lower	
Hydra-DPX-TPP-HA-NPs	In vitro on MCF-7/ADR cells	Cancer cell death	72%	50%	[61]
PTX-HA-NPs	In vitro on A549 cells	Cellular uptake	4.8 fold increased		[62]
		Cancer cell cytotoxicity (1μg/ml)	40%	60%	
		ATP concentration	70%	80%	
		Relative caspase 3 activity	1.7	1.4	
DOX@BHNP	In vitro on 4T1 cells, in vivo on 4T1 tumor-bearing mice	Apoptosis induction	60%	25%	[75]
		Bcl-2 expression	Higher	Lower	
		Cleaved caspase 3 expression	Higher	Lower	
		Cellular uptake specific to tumor site	35%	15%	

For instance, in an in vitro study conducted by Hsiao et al. [28], Sinulariolide-Conjugated HA-NPs demonstrated a substantial increase in apoptosis in lung adenocarcinoma cells, reaching 50.1% compared to 12.8% for non-coated SLN. Additionally, there was a significant reduction in cellular viability, indicating the potency of HA-SLN in inhibiting cellular proliferation. Even at lower concentrations, HA-SLN showed superior efficacy in reducing cellular migration, suggesting its potential for suppressing metastasis. Moreover, HA-SLN induced a higher degree of cell cycle arrest at the S-G1 phase, registering 9.2% compared to 3.5% for naked SLN. Higher levels of caspase expression imply HA-SLN's capacity to enhance programmed cell death pathways, bolstering its anti-cancer effects. Notably, HA-SLN displayed fewer side effects, promising a more tolerable treatment option for patients. This comprehensive data underscores HA-SLN's potential as a promising therapeutic strategy against cancer, offering both heightened efficacy and improved safety profiles [28].

Another recent study showed in vitro that the presence of HA in PEG modified barberine derivative (HA-PEG BD) NPs increases the size of the nanoparticles enhancing their stability and ability to reach target sites [32]. This improved stability also contributes to better selectivity and more consistent drug delivery. (HA-PEG BD) NPs demonstrated a more gradual drug release, with 20% of the drug released after 25 hours compared to 30% without HA, leading to a longer therapeutic effect. Additionally, HA-NPs significantly reduced the cancer cell survival rate to 40% at a concentration of 5 μg/ml, compared to 60% without HA. They also decreased the mitochondrial membrane potential more effectively, with a reduction to 50% with HA compared to 30% without HA [32].

The anti-tumor activity of the treatment was markedly enhanced with HA-NPs, as evidenced by the higher level of apoptosis (50.3% for HA-NPs vs. 39.7% for NPs). Furthermore, HA-NPs improved mitochondrial targeting of the drug by approximately 10%. There was also an increased ratio of caspase activity, with caspase 9 activity rising from 1.2 without HA to 1.5 with HA, and caspase 3 activity increasing from 1.4 to 1.6 with HA. Additionally, HA-NPs enhanced the expression of pro-apoptotic Bax and reduced the expression of anti-apoptotic Bcl-2 [32].

The enhanced performance of HA-NPs in cancer treatment compared to naked NPs was also shown in breast cancer cells [37]. Cerium oxide (CePEI) HA-NPs demonstrated higher cytotoxicity, achieving about 50% more effectiveness in killing cancer cells. Additionally, there was an approximately fivefold increase in cellular uptake with HA-NPs, highlighting their superior ability to deliver the therapeutic agent directly to the cancer cells [37].

In this sense, Wang et al. [38] demonstrated in vitro that HA-camptothecin-NPs significantly enhance the efficacy and safety of cancer treatment compared to not coated NPs. The enhanced ability of HA-NPs to reach target sites led to improved therapeutic outcomes. Cellular uptake was notably higher with HA-NPs, showing a twofold increase for MCF-7 cells, an eightfold increase for HepG2 cells, and a fourteenfold increase for MDA-MB-231 cells [38]. This enhanced uptake translated into higher cytotoxicity in cancer cells, with about a 12% increase for MCF-7 cells, and a 20% increase for both HepG2 and MDA-MB-231 cells. Importantly, HA-NPs exhibited lower cytotoxicity in normal cells, reducing it by approximately 50%, which highlights their selectivity and safety [38]. Additionally, HA-NPs reduced cell migration by about 10%, which could potentially limit metastasis. The increased expression of pro-apoptotic proteins, with a 1.5-fold increase for Bax and a 2.6-fold increase for P53, along with a 70% reduction in the expression of the anti-apoptotic protein Bcl-2, further supported the pro-apoptotic and anti-cancer effects of HA-NPs [38].

Similarly, TPP-DOX-HA-phenyl boronic acid pinacol ester (PBPE) showed promise in enhancing cytotoxicity towards cancer cells by 7.3 times [40].

In another recent in vitro study, HA-folic acid (FA)- BD-NPs exhibited higher cellular uptake, approximately 24% more than NPs without HA. This increased uptake enhanced the delivery of the therapeutic agent directly to cancer cells, improving the treatment's effectiveness [41]. Additionally, HA-NPs led to an increase in apoptosis by about 10%, further supporting their role in promoting cancer cell death. The cytotoxicity of HA-NPs was also notably higher, with an increase of approximately 40%, indicating a greater ability to kill cancer cells compared to NPs without HA [41].

Moreover, Pan et al. [43] showed several key benefits of gold nanostars-dendritic polyglycerol (GNSs-dPG) 3-bromopyruvate (BP)-TPP- HA NPs in cancer treatment compared to NPs without HA. HA-NPs exhibited a significant increase in cytotoxicity, approximately 25% higher than NPs without HA, enhancing their ability to kill cancer cells effectively [43]. Additionally, HA-NPs showed higher suppression of stemness-associated genes, crucial for preventing cancer cell proliferation and resistance [43].

The PTT was also enhanced with HA-NPs, reaching 55°C compared to 48.9°C without HA, indicating a more efficient heat-induced tumor cell destruction. This increased PTT contributed to greater necrosis and apoptosis of tumor cells, further supporting their anti-cancer efficacy [43]. Moreover, HA-NPs led to a reduced expression of key stemness-associated genes: OCT4 expression decreased from 40% without HA to 30% with HA, NANOG expression dropped from 40% without HA to 15% with HA, and SOX2 expression was reduced from 40% without HA to 25% with HA. These reductions highlight the potential of HA-NPs to target and diminish the cancer stem cell population, potentially reducing recurrence and improving long-term treatment outcomes [43].

Another presented compelling evidence of the enhanced efficacy of HA-NPs in cancer treatment compared to naked NPs. HA and geraniol linked via a disulfide bond linker (HA-ss-Geraniol NPs) demonstrated increased cytotoxicity, indicating a more potent effect in eliminating cancer cells [50]. Moreover, HA-NPs resulted in an eightfold increase in apoptosis, significantly promoting the programmed death of cancer cells and thus improving the overall effectiveness of the treatment. This heightened apoptosis was a key indicator of the therapeutic potential of HA-NPs. Additionally, HA-NPs induced a higher rate of cell cycle arrest, with 21.97%

of cells arrested compared to 9.00% without HA. This substantial increase in cell cycle arrest further underscored the ability of HA-NPs to inhibit cancer cell proliferation [50].

HA-NPs demonstrated greater additionally cellular targeting, which enhanced the delivery of the therapeutic agent directly to cancer cells. This improved targeting was reflected in the lower IC50 value of 8.0 μg/mL for HA-coated NPs, compared to 13.1 μg/mL for non-coated NPs, indicating higher potency [51]. Furthermore, HA-NPs resulted in a significant reduction of cell viability in A549 cells. For a concentration of 5 μg/mL after 24 hours of treatment, cell viability dropped from 43% without HA to 22% with HA, showcasing the increased effectiveness of HA-NPs in inhibiting cancer cell growth. Better mitochondrial targeting was another critical benefit of HA-NPs, which enhanced their therapeutic efficacy. HA-NPs also led to enhanced production of ROS, which contributed to increased apoptosis induction. The expression of pro-apoptotic proteins such as caspase 9, caspase 3, and cytochrome c was increased with HA-NPs, further promoting programmed cell death. Concurrently, the expression of the anti-apoptotic protein Bcl-2 was reduced, facilitating the apoptotic process [51].

Several studies [52, 57] demonstrated that HA-NPs led to increased apoptosis induction, as evidenced by changes in key apoptotic markers, such as decreased cytochrome c ratio and increased caspase 3 ratio. Yu et al. indeed showed that the cytochrome c ratio decreased from 0.6 without HA to 0.4 with HA, and the caspase 3 ratio increased from 0.5 without HA to 0.7 with HA [52].

Furthermore, HA-NPs achieved higher levels of cell death even at lower drug concentrations (cytotoxicity increased from 20% without HA to 35% with HA), complemented by improved vitality of healthy cells (cell viability of health cells increased from 60% without HA to 90% with HA) [57].

Moreover, HA-NPs significantly inhibited the migration of cancer cells, potentially reducing the metastatic spread of cancer (the number of migratory cells dropped from 60% without HA to 10% with HA). They also amplified the loss of mitochondrial membrane potential, a crucial step in the induction of apoptosis in cancer cells [57].

Furthermore, HA-NPs led to increased expression levels of mitochondrial apoptotic proteins and caspase-dependent proteins, such as cleaved caspase-3 and caspase-9, which were key regulators of apoptosis. This resulted in a higher induction of early apoptosis (increase from 12% without HA to 25% with HA) and an overall increase in the rate of apoptosis in cancer cells treated with HA-NPs (apoptosis rate increased from 12% without HA to 35% with HA) [57].

In one study [61], hydra-DPX-TPP- HA-NPs showed a notable increase in drug release at acidic pH values, leading to elevated cellular uptake and significantly higher cytotoxicity in cancer cells. Specifically, the inclusion of HA resulted in a remarkable 72% cell death rate compared to 50% in treatments without HA.

Another study [62] focused on the efficacy of PTX loaded HA-liposomes, revealing a significant 4.8-fold increase in cellular uptake compared to conventional methods. Moreover, the system exhibited improved mitochondrial targeting, resulting in increased inhibition of ATP production. HA-PTX&DQA liposomes demonstrated a remarkable decrease in ATP production to about 40% compared to other groups, along with enhanced apoptosis induction, indicating its potential for improving cancer therapy.

Furthermore, in a study DOX loaded in a novel biotinylated HA-guided dual-functionalized $CaCO_3$-based NPs (DOX@BHNP) showed an increased apoptosis induction from 25% (without HA) to 60%, indicating enhanced cancer cell death [75]. It also led to lower expression of Bcl-2, a protein associated with cell survival, and higher expression of cleaved caspase 3, a marker of apoptosis activation, further highlighting its efficacy in combating cancer cells [75].

In in vivo studies, the efficacy of HA in cancer treatment has also been demonstrated. The HA-PEG BD NPs were effective in slowing the increase in tumor volume (by approximately 50%) and achieving a tumor inhibitory rate exceeding 35% than the approach without HA [32].

In enhancing the accumulation of the antitumoral drug inside the tumor site, TPP-DOX-HA-PBPE was more effective than NPs without HA [40]. Furthermore, HA-FA BD NPs demonstrated a greater reduction in tumor volume (about 60%), a tumor inhibitory rate exceeding 30%, and a reduced number of lung metastatic nodules by about 70% compared to NPs without HA [41]. Similarly,

GNSs-dPG-3BP-TPP-HA was also more effective in reducing tumor growth than the naked NPs [43]. Additionally, HA-ss-Geraniol NPs caused an enhanced tumor growth inhibitory ratio (54.0% with HA, 9.2% without HA) [50]. Moreover, Hydra-DPX-TPP-HA-NPs halved the tumor volume (150 mm3 without HA, 90 mm3 with HA) [61]. Lastly, DOX@BHNP demonstrated higher cellular uptake, improving from 15% to 35% specifically at the tumor site, thereby enhancing drug delivery precision [75].

Studies have consistently demonstrated the superiority of HA-coated nanoparticles in terms of cellular uptake, cytotoxicity, apoptosis induction, and inhibition of cancer cell migration compared to non-coated nanoparticles. Moreover, HA-NPs exhibit enhanced stability, improved mitochondrial targeting, and reduced side effects, making them promising candidates for cancer treatment. In vivo studies further support the effectiveness of HA-based drug delivery systems in tumor inhibition and metastasis prevention. Overall, the comprehensive evidence presented underscores the pivotal role of HA in advancing cancer therapy, offering a targeted, efficient, and safer approach to combatting this complex disease.

3.4 Validating data on patients

The presence of HA in drug delivery systems offers several advantages for targeted cancer therapy. Firstly, HA has a high affinity for CD44 receptors, which are often over-expressed on the surface of cancer cells. This allows drug-loaded HA systems to specifically target cancer cells while sparing healthy cells, reducing the risk of side effects commonly associated with chemotherapy. Additionally, HA can enhance the delivery of drugs to specific subcellular compartments, such as the mitochondria. Targeting the mitochondria is particularly important in cancer therapy because mitochondria play a crucial role in cell survival and proliferation. By delivering drugs directly to the mitochondria, HA-based drug delivery systems can disrupt mitochondrial function and induce apoptosis in cancer cells, thereby inhibiting tumor growth.

Moreover, HA-based drug delivery systems can improve the pharmacokinetics of anticancer drugs by enhancing their stability, solubility, and bioavailability. This ensures that a sufficient amount of the drug reaches the tumor site, increasing therapeutic efficacy.

Overall, the incorporation of HA into drug delivery systems holds great promise for improving the effectiveness and selectivity of cancer treatments while minimizing adverse effects on healthy tissues.

To assess the true effectiveness of healthcare interventions involving HA (healthcare interventions), a systematic analysis of clinical trials was conducted. The search included reputable databases such as clinicaltrials.gov and Cochrane, resulting in the identification of four relevant studies, which comprised two previously identified by MySLR platform [31, 102] and two additional ones [NCT04798703, 29]. Following this selection process, a comprehensive analysis of the clinical trial data was carried out to verify their practical applicability and relevance in real-world settings.

Regarding the expression of CD44 receptors as a potential benefit for cancer therapy, an example is Oncofid® (Fidia Farmaceutici SpA, Abano Terme, Italy), which is a drug composed of HA conjugated with paclitaxel (taxol) a chemotherapeutic used in the treatement of treat several patients with bladder carcinoma in situ (CIS).

In a recent phase I exploratory study, the safety, tolerability, and antitumor activity of ONCOFID-P-B™ therapy were evaluated in adult patients with CIS who were unresponsive or intolerant to Bacillus Calmette-Guérin (BCG) therapy. The study involved 20 patients who initially received 12 weekly intravesical instillations of ONCOFID-P-B™ during the intensive treatment phase. Patients who achieved a complete response (CR) after the 12 weekly instillations entered the maintenance phase of the study, where ONCOFID-P-B™ was administered once a month for 12 months [NTC04798703].

At the end of the intensive phase, 15 out of the 20 enrolled patients (75%) achieved a complete response. The percentages of patients still in complete response after 3, 6, 9, and 12 months of the maintenance phase were 65%, 60%, 45%, and 40%, respectively. Only seven drug-related adverse events (five mild and two moderate) were reported in three patients. No drug-related serious adverse

events or withdrawals were reported. The drug concentrations in all plasma samples were below the lower limit of quantification (1 ng/ml) [102].

This study demonstrates the potential effectiveness and safety of ONCOFID-P-B™ therapy in treating patients with bladder carcinoma in situ who were unresponsive or intolerant to BCG therapy.

Another trial, involving 34 patients with extensive stage small cell lung cancer (ESLC), aimed to assess the efficacy of HA in delivering irinotecan to selectively target CD44 expressing tumor cells. Although no significant difference in progression-free survival (PFS) was observed between the treatment arms, the overall response rate was encouraging at 50%, with 12% experiencing complete responses and 38% showing partial responses. However, it demonstrated both tolerability and efficacy, with a lower incidence of grade III/IV diarrhea. These findings highlight the potential of HA-irinotecan as a promising therapeutic approach in SCLC treatment, suggesting the need for further investigation into its role in improving patient outcomes [29].

Additionally, a recent study focused on the use of HA in SCLC treatment, highlighting the potential significance of HA in enhancing therapeutic outcomes. Among the 39 patients screened, 34 were eligible for the study, underlining the feasibility and relevance of the research in a clinical setting. The median age of the included patients was 66, with a range from 39 to 83 years old [30].

One of the key findings of this study was the comparable tumor response rates between the experimental, HA-irinotecan (IR) and carboplatin (C) HA-IR+C, and standard (irinotecan and carboplatin IR + C) treatment arms, indicating HA's effectiveness in enhancing treatment outcomes across different age groups and demographic backgrounds. Furthermore, the observed median PFS of 42 weeks in the experimental arm further supports HA's potential as a therapeutic agent in SCLC management [30].

Importantly, the safety profile of HA-IR+C was favorable, with well-tolerated treatments reported across the study cohorts, including patients of varying ages and clinical histories. Notable differences in adverse events between the experimental and standard arms underscore HA's potential in mitigating specific treatment-related toxicities. For instance, the higher incidence of grade III/IV diarrhea observed in the standard arm compared to anemia, which was more common in the experimental arm, suggests a potential advantage of HA-IR+C in terms of tolerability [30].

Given the efficacy of HA in anticancer therapy, further studies are warranted to assess therapy efficacy in larger patient populations and across different cancer types. However, transitioning HA-based drug delivery systems from laboratory research to clinical practice faces several significant barriers, including regulatory challenges, scalability and manufacturing issues, and complex clinical trial design. Additionally, securing funding, understanding biological variability among patients, integrating with existing therapies, and gaining market acceptance are crucial for successful implementation. Long-term safety and efficacy monitoring will also be essential to ensure positive patient outcomes. The implementation of clinical trials will undoubtedly be a focal point for future research, addressing these challenges and paving the way for effective HA-based therapies.

4. Discussion

The research presented highlights the significant potential of cancer therapies based on HA drug delivery systems, supported by a comparative analysis of the efficacy of HA-coated nanoparticles versus non-coated nanoparticles in cancer treatment, along with a dataset of clinical trials and machine learning analysis. The results of the analyzed clinical trials clearly demonstrate that the incorporation of HA into drug delivery systems offers numerous advantages in cancer therapy. Firstly, HA exhibits high affinity for CD44 receptors, which are often overexpressed on the surface of cancer cells, allowing HA-loaded drug delivery systems to specifically target cancer cells, thus reducing the risk of side effects associated with traditional chemotherapy. The role of CD44 is extremely positive in this context, as its overexpression on a wide variety of tumour cells is exploited: its ligand, with which the nanoparticles are functionalized, allows for greater selectivity and a reduction in side effects. Additionally, HA facilitates the delivery of drugs to specific subcellular compartments, such as mitochondria, which are crucial in cell survival and proliferation, thereby increasing therapeutic efficacy and reducing toxicity to healthy cells (Fig. 5).

However, achieving precise mitochondrial targeting while minimizing adverse effects on healthy tissues poses a significant challenge [103, 104]. Nonetheless, the use of HA represents a valuable tool to overcome these limitations. Thanks to its unique properties, HA offers characteristics that facilitate the targeting of drugs to mitochondria, while maintaining greater selectivity towards cancer cells compared to healthy tissues. This innovative HA-based approach continues to show remarkable progress in research, opening new prospects for improving the efficacy and reducing the toxicity of cancer therapies.

This means that drug delivery systems based on HA offer several unique advantages that make them a promising approach for cancer treatment. It was described that HA can facilitate the intracellular delivery of drugs by promoting their uptake into cancer cells. Moreover, HA is a naturally occurring, biocompatible, and biodegradable polymer, which makes it an attractive material for developing safe and well-tolerated drug delivery platforms. The versatility of HA allows for the development of various nanoparticles with the potential to optimize the pharmacokinetics and pharmacodynamics of the encapsulated therapeutic agents.

Overall, the unique properties and targeting capabilities of HA-based drug delivery systems, as highlighted in the research, contribute to their significant potential for improving the effectiveness and safety of cancer therapies.

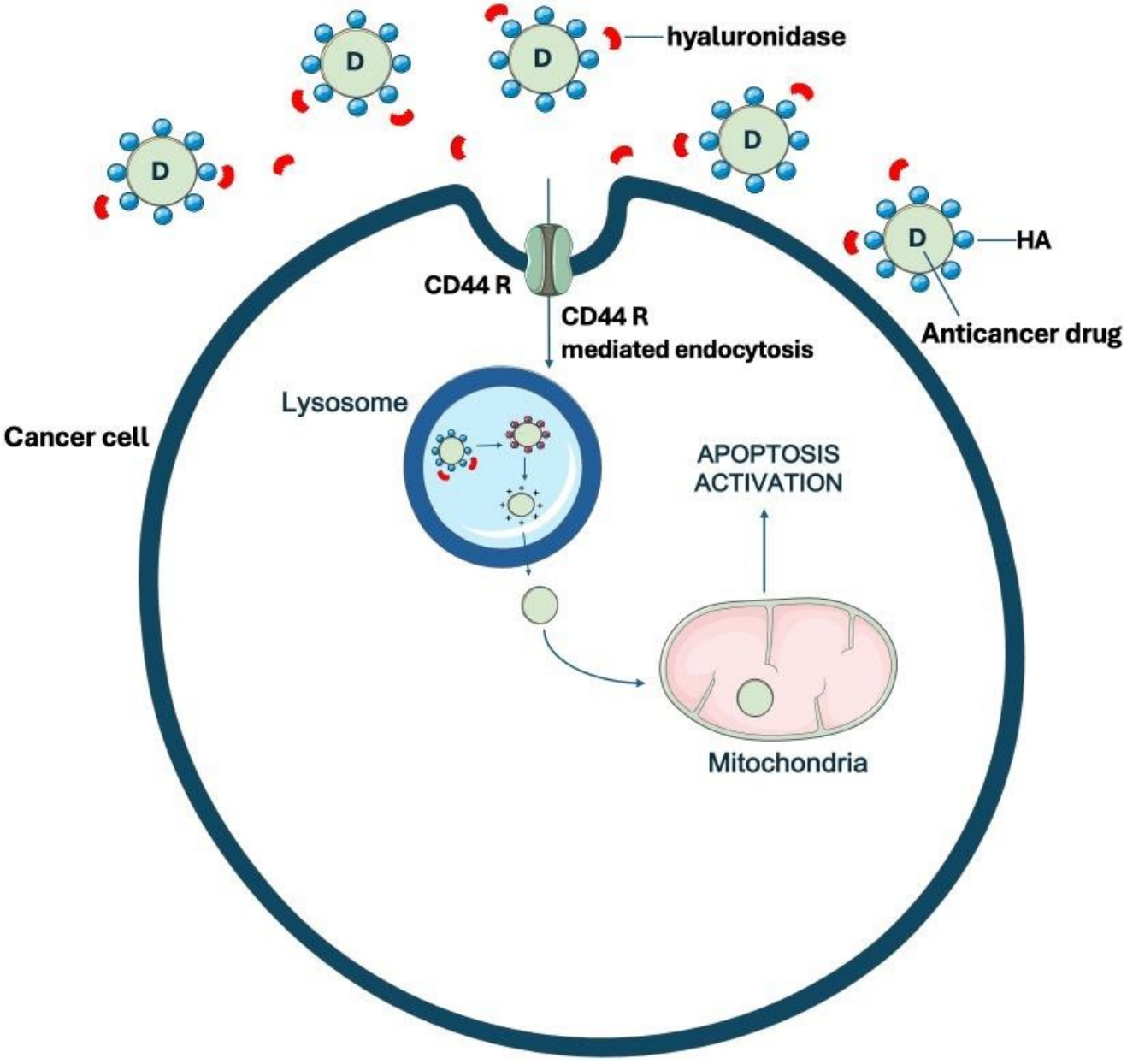


Figure 5. Mechanism of Action of HA-Based Drug Delivery System Targeting Mitochondria. This image depicts the process by which a HA-based drug delivery system is firstly degraded by HYAL and then endocytosed in cancer cells via CD44 receptors,

which are often overexpressed on the cell surface. Upon entry, the drug-loaded HA system undergoes degradation within the lysosomes of the cell mediated by the acidic pH and the HYAL activity. Due to its positive charge, the drug delivery system is able to escape the lysosomal compartment and target the mitochondria within the cell. This targeted delivery to the mitochondria disrupts their function, leading to apoptosis in cancer cells and inhibiting tumor growth.

Analyses conducted through machine learning provide an in-depth understanding of the relationships between different topics and the characteristics of patients involved in clinical trials.

Utilizing algorithms like LDA integrated into platforms such as MySLR streamlines data analysis, identifying key themes within scientific literature and guiding researchers toward emerging trends and patterns.

By employing advanced analytical tools like Machine Learning Analysis, researchers can uncover nuanced insights into the impact of HA on drug delivery systems targeting mitochondria for cancer therapy. This approach enhances our understanding of mechanisms, efficacy, and potential areas for further research, potentially leading to new avenues for innovation and treatment optimization beyond existing reports.

The use of the LDA algorithm facilitated the screening and categorization of 90 works into two distinct topics, each describing different yet interconnected strategies.

The first one highlights diverse targeting strategies and biological actions of HA in cancer therapy, focusing on enhancing drug delivery, tumor targeting, and therapeutic efficacy (Table 1). Research reveals the significant overexpression of CD44 receptors in various cancer types, enabling HA's use as a targeting moiety to deliver therapeutic agents exclusively to cancer cells, maximizing drug accumulation within tumors while minimizing adverse effects on healthy tissues.

Strategies focusing on mitochondrial targeting aim to enhance treatment effectiveness by selectively attacking cancer cell energy metabolism and survival pathways. Additionally, engineering nanoparticles to respond to stimuli in the tumor microenvironment allows for selective drug release, improving therapeutic outcomes while reducing systemic toxicity.

The unique viscoelastic properties of HA facilitate its penetration deep into tumor tissues, overcoming barriers such as dense extracellular matrix and heterogeneous blood flow. This property enhances the distribution and penetration of therapeutic agents within solid tumors, optimizing treatment efficacy.

Table 2 provides an overview of various types of HA-based drug delivery systems, showcasing their versatility and potential in biomedical applications. These systems encompass a wide array of applications, ranging from subcellular targeting to combined therapy and overcoming multidrug resistance. Each category highlights specific strategies for enhancing drug delivery, tumor targeting, and therapeutic efficacy, underscoring the multifunctional capabilities of nanoparticles in cancer therapy.

By analyzing the selected studies in detail, it becomes evident that incorporating HA in drug delivery systems offers significant advantages (Table 3). HA-NPs have shown a marked reduction in cancer cell viability, effectively inhibiting cancer cell growth. The inclusion of HA improves mitochondrial targeting, increases ROS production, and enhances apoptosis induction through the upregulation of pro-apoptotic proteins and downregulation of anti-apoptotic proteins compared to using naked NPs. This enhancement is beneficial not only for cancer therapy, as detailed in the studies, but also for other pathological conditions [105].

It has been described that HA-NPs exhibited also increased cytotoxicity towards tumor cells, achieving higher levels of cell death even at lower drug concentrations compared to NPs. This heightened cytotoxicity was complemented by improved vitality of healthy cells. Additionally, HA-NPs significantly inhibited the migration of cancer cells, potentially reducing metastatic spread.

Studies on different HA-NP formulations (Table 3), such as hydra-DPX-TPP-HA-NPs and HA-liposomes loaded with PTX, demonstrated elevated cellular uptake, increased cytotoxicity in cancer cells, and enhanced apoptosis induction, highlighting their potential in cancer therapy. Additionally, these formulations showed notable increases in drug release at acidic pH values, leading to higher cellular uptake and significantly greater cytotoxicity. Studies on DOX loaded in HA-guided dual-functionalized NPs and

HA-FA-BD-NPs demonstrated higher cellular uptake, increased apoptosis, and notable cytotoxicity towards cancer cells, indicating their potential in improving cancer therapy.

Also in vivo studies highlighted the efficacy of HA-based NPs in cancer treatment, showing reductions in tumor volume, tumor inhibitory rates, and enhanced accumulation of antitumoral drugs inside tumor sites. Particularly, HA-PEG BD NPs and TPP-DOX-HA-PBPE were effective in slowing tumor growth and improving drug accumulation at the tumor site.

These results collectively demonstrate the potential of HA-based NPs in enhancing targeted drug delivery, improving therapeutic outcomes, and reducing side effects in cancer therapy. The studies discussed in the document provide valuable insights into the efficacy and mechanisms of action of HA-NPs in combating cancer cells.

Furthermore, the results of clinical trials on patients with bladder carcinoma in situ and SCLC demonstrate that the use of HA-based therapies, such as ONCOFID-P-B™ and HA-irinotecan, leads to a significant reduction in disease progression and improved tolerability compared to standard therapies. Furthermore, the consistency of results across trials on different patient groups underscores the universality of the effectiveness of these therapies.

So the results of our study highlight the importance of targeting the CD44 receptor and inhibiting HA synthesis in the context of cancer therapy. We observed a significant increase in apoptosis induction in cancer cells, consistent with previous studies demonstrating that the apoptotic pathway can be mediated by the interaction between HA and CD44 [106, 107]. Specifically, our findings align with those of Della Sala et al. (2022), who reported similar apoptotic effects when HA was utilized in anti-tumoral therapies. This reinforces the notion that CD44 plays a pivotal role in mediating apoptosis through HA interactions.

In our research, we focus on an innovative aspect: HA based mitochondrial targeting. Our analysis of cell cycle arrest highlights a significant block in the G1 and G2/M phases, which may be attributed to enhanced mitochondrial targeting. Additionally, our investigation of the expression and activity of caspases shows increased activity of caspases 3 and 9, crucial for apoptosis. This finding supports previous research highlighting the role of caspases in the apoptotic processes induced by HA-targeting strategies [100].

Moreover, our works describe how HA-based drug delivery system not only enhances drug accumulation at the tumor site but also induces a loss of mitochondrial membrane potential, a key indicator of mitochondrial stress and apoptosis. These results align with studies emphasizing the critical role of mitochondria in regulating cell survival in tumors, suggesting that the presence of HA may enhance therapeutic efficacy by directly impacting mitochondrial function.

In terms of side effects on healthy cells, our research indicates a significant reduction in toxicity when HA is present compared to traditional chemotherapy regimens, confirming observations from other authors who emphasized the selectivity of CD44 targeting [107]. This selectivity is particularly relevant for the development of safer and more targeted therapies, as highlighted in the comparative analyses of HA-based therapies versus conventional treatments.

Finally, the analysis of the number of migratory cells revealed a significant inhibition of cell migration, suggesting that the presence of HA not only reduces tumor growth but may also limit metastasis, a crucial aspect in cancer management. This finding is in line with previous studies that have documented the anti-metastatic properties of HA in various cancer models.

In conclusion, our work supports the idea that the integration of HA into drug delivery systems represents a promising strategy to enhance the effectiveness of cancer therapies while simultaneously reducing side effects. However, further studies on a larger scale and across different tumor types are needed to confirm and optimize such therapeutic approaches.

5. Conclusion

In conclusion, the integration of HA into drug delivery systems represents a promising approach to enhance the effectiveness and safety of cancer therapies.

The scientific works analyzed delved into various parameters to gauge the impact of HA on drug delivery systems.

One crucial aspect was the specificity of targeting, examining HA's ability to target CD44 receptors on cancer cells. Additionally, researchers evaluated HA's subcellular targeting capabilities, assessing its capacity to deliver drugs to specific compartments within cancer cells, such as the mitochondria, thereby disrupting cellular functions and inducing apoptosis to inhibit tumor growth.

Another focus was on assessing the impact of HA on the stability, solubility, and bioavailability of anticancer drugs. Enhancing the pharmacokinetic properties of these drugs ensures optimal delivery to the tumor site, thereby increasing therapeutic efficacy.

Biocompatibility of HA-based drug delivery systems was also scrutinized, ensuring minimal toxicity and adverse effects on healthy tissues, which is crucial for successful clinical translation and patient care.

Furthermore, the capacity of HA to serve as a carrier material for loading and efficiently delivering anticancer drugs played a pivotal role. This capability significantly influences the effectiveness of the drug delivery system in targeting cancer cells.

Researchers also likely examined the efficiency of cellular uptake of HA-based drug delivery systems and the subsequent intracellular release of the drugs. Understanding these mechanisms can influence the overall efficacy of drug delivery to cancer cells.

Lastly, evaluating the overall impact of HA on enhancing the therapeutic efficacy of anticancer drugs was critical. Assessing the ability of HA-based systems to inhibit tumor growth, induce apoptosis, and improve patient outcomes is essential for determining their effectiveness in cancer treatment.

The clinical trials discussed have provided compelling evidence of the potential of HA-based treatments in targeting cancer cells while minimizing adverse effects on healthy tissues. Moreover, machine learning analysis has contributed valuable insights into patient characteristics and treatment outcomes, aiding in the optimization of therapeutic strategies.

The results from trials focusing on CIS and SCLC highlight the significant clinical benefits of HA-based therapies, including improved response rates and tolerability profiles compared to standard treatments. These findings underscore the importance of further research to validate the efficacy of HA-based therapies across diverse patient populations and cancer types.

While this study makes significant contributions, certain limitations should be noted:

- The LDA algorithm may have included irrelevant articles, necessitating a human review for accuracy in selection.
- Biases during the human review process could lead to subjective interpretations, affecting consistency in study relevance.
- Focusing solely on studies published in the last ten years may exclude valuable insights from older research.

To build upon these findings, future studies should:

1. Combine automated algorithms with human review to enhance the selection process.
2. Expand the scope of literature reviewed to include older studies, enriching the analysis.
3. Investigate additional biological mechanisms of HA in cancer treatment.
4. Foster interdisciplinary collaboration to refine methodologies and promote clinical translation.
5. Utilize advanced machine learning techniques to gain deeper insights from scientific literature.

Overall, this study supports the ongoing exploration and development of HA-based drug delivery systems in cancer therapy. The advancements in biomaterials and targeted delivery through nanotechnology inspire optimism for overcoming the limitations of conventional chemotherapy. As clinical research and machine learning techniques advance, there is substantial potential to unlock new therapeutic avenues and improve outcomes for cancer patients.

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