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REVIEW ARTICLE

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State-of-the-Art of the Major Nanopharmaceuticals Treatments in Breast Cancer: A Systematic Review

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Abstract: **Introduction:** Breast cancer is the most frequently detected cancer in women worldwide, its metastasis is responsible for 90% of deaths. Breast carcinoma is the most common cancer in women worldwide and the most common cause of deaths associated with malignancies. Hyaluronic acid (HA) is the main molecule binding to CD44 and has proved to be a significant ally in the development of nanotransporters that demonstrate preferential accumulation of tumors and increased cellular uptake. **Objective:** Carry out a systematic review of the main treatments to reduce or prevent the proliferation of breast cancer. **Methods:** A total of 59 articles have found and after the selection process 20 articles have included and discussed in this study. PUBMED, EMBASE, OVID, AND COCHRANE LIBRARY databases were searched. **Results:** cationic liposomes containing the conjugate hyaluronic acid-dioleoylphosphatidylethanolamine (HA-DOPE) mediated good transfection in cell lines that express CD44 in culture. Still, other results suggested that the formulation of lapatinib (LPT) coated with HA increases the activity of LPT against triple-negative breast cancer. In addition, compared to free doxorubicin (DOX), superior in vivo antitumor efficacy of modified carbon spots (HA HA-CD) and (p-CBA-DOX) was observed in heterotopic and orthotopic 4T1 cell tumor models. In addition, hematological and biochemical analysis of blood showed that HA-CD and p-CBA-DOX did not induce noticeable toxicity, which further confirmed the good biocompatibility of HA-CD and p-CBA-DOX. Also, it was found that CD44v expression can negatively influence HA uptake and, instead, when cells expressed mainly CD44s, a positive correlation between expression and uptake was observed. Other findings point to the potential clinical utility of recombinant human proteoglycan 4 (rhPRG4) as a therapeutic treatment for invasive and metastatic breast cancer. **Conclusion:** The development of nanopharmaceuticals delivery systems are able to control the development of tumors and represent a promising strategy to overcome issues related to the non-specific effects of conventional anticancer therapies.

Keywords: Breast cancer, Hyaluronic acid, CD44, Gene therapy, Nanopharmaceuticals, New treatments.

1. Introduction

Breast cancer is the most frequently detected cancer in women worldwide [1], and metastasis is responsible for 90% of deaths [2]. Breast carcinoma is the most common cancer in women worldwide and the most common cause of deaths associated with malignancies. In this scenario, the development of new therapies aimed at combating various types of cancer was made possible by the discovery of altered components of the extracellular or intracellular membrane in cancer cells compared to normal cells [3].

In the case of breast cancer, therapies that bind and inhibit the function of the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor (HER2) have led to increased survival rates for patients whose tumors they are characterized as positive ER, positive PR and amplified HER2, respectively [4]. Breast tumors that are ER and PR negative, without HER2 amplification, are termed as triple-negative breast cancer (TNBC) [4].

In this sense, TNBC represents only 10 to 20% of all diagnosed breast cancer cases, but are highly metastatic and associated with poor prognosis and worse mortality rates compared to other molecular breast cancer subtypes [4]. The treatment of TNBCs is mainly limited to conventional chemotherapeutics that

are associated with significant adverse effects, with recurrence of tumors [5]. Thus, new therapies that can reach the TNBC subtype with reduced or no toxicity has needed in the treatment of cancer [6].

In this scenario, the differentiation group-44 (CD44) is a glycoprotein present on the surface of mammalian cells that plays a significant role in various biological functions [7]. Since the discovery that the receptor is overexpressed in a variety of solid tumors, such as pancreatic, breast and lung cancer, many studies have focused on methods to target CD44 in an attempt to improve drug delivery and discrimination between healthy tissues and malignant, while reducing residual toxicity and off-target accumulation. Related to this, hyaluronic acid (HA) is the binding molecule to CD44 has proven to be a significant ally in the development of nano-transporters that demonstrate preferential accumulation of tumors and increased cell uptake [7-10].

Recent experimental and clinical evidence shows that HA and the CD44 receptor regulate the proliferation, migration, and invasion of cancer cells, as well as tumor-associated angiogenesis and has correlated with patient survival. These results suggest that they can has used as prognostic factors and goals for the treatment of breast cancer [11-13].

Therefore, as HA with CD44 contributes significantly to cell proliferation and migration and may also be involved in the progression of some malignant tumors, the present study aimed to conduct a systematic review of the treatments to reduce or prevent the proliferation of breast cancer.

2. Methods

2.1 Data sources and search strategy

A total of 59 articles were found involving the MeSH Terms: *Breast cancer. Hyaluronic acid. CD44. Gene therapy. Nanopharmaceuticals. New treatments.* Initially, it was held the existing exclusion title and duplications following the interest described in this work. After this process, 20 articles were included and discussed in this study (Figure 1). PUBMED, EMBASE, OVID AND COCHRANE LIBRARY databases have searched. Initially, the descriptors were determined by searching the DeCS tool and later verified and validated by the MeSH Terms System (US National Library of Medicine).The present study has elaborated according to the rules of systematic review- PRISMA (Transparent reporting of systematic reviews and meta-analyses- http://www.prisma-statement.org/).

2.2 Study selection and risk of bias in each study

Two independent reviewers (1 and 2) performed research and study selection. The data extraction was performed by reviewer 1 and fully reviewed by reviewer 2. A third investigator decided some conflicting points and made the final decision to choose the articles. Only studies reported in Portuguese and English were evaluated. The **Cochrane instrument** was adopted to assess the quality of the included studies.

2.3 Risk of bias

Considering the Cochrane tool for risk of bias, the overall evaluation resulted in 3 studies with a high risk of bias and 3 studies with uncertain risk. Five studies had a limited number of participants. Also, the absence of the source of financing in 2 studies. Further, 1 studies did not disclose the information on the conflict of interest statement.

3. Development and Discussion

HA contributes to tissue hydrodynamics, cell movement, and proliferation and participates in various interactions with cell surface receptors, especially those that include their primary receptors, CD44 and receptor for hyaluronan-mediated motility (RHAMM) [2]. Positive regulation of CD44 itself is widely accepted as a marker of cell activation in lymphocytes. The contribution of HA to tumor growth may be due to its interaction with CD44. The CD44 receptor participates in the cell adhesion interactions required by tumor cells [3-5].

The CD44 antigen is a cell surface glycoprotein involved in cell-cell interactions, cell adhesion, and migration. In humans, the CD44 antigen is encoded by the CD44 gene on the chromosome [6,7].

The HA performs different functions, such as lubrication, hydration, and maintenance of the tissue structure. In addition, it is involved in cell proliferation and migration events, as well as angiogenesis. In solution, HA has a gelatinous consistency with high viscoelasticity and a high degree of hydration due to the structural characteristics of the molecule [8]. HA interacts with water through hydrogen bonds, giving the polymer a high water holding capacity and conformational rigidity [9].

Thus, the main receptor for HA is CD44, a family of transmembrane glycoproteins encoded by a

single gene with at least 19 exons that map to the 11p13 band on the human chromosome.

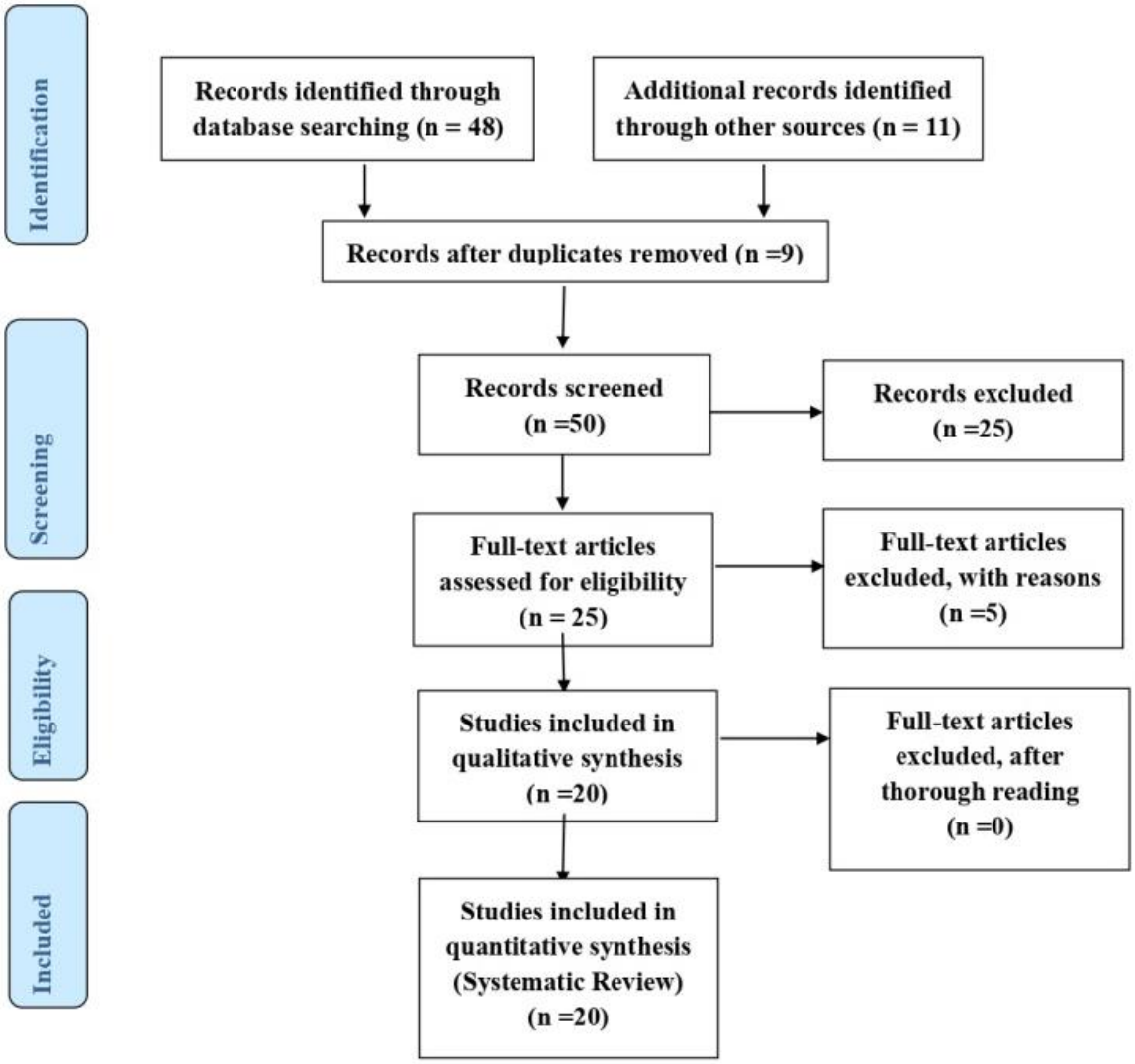

Figure 1 Flow Chart of the article selection process.

The level of cytosolic HA defines some clinical-biological properties of the infiltrating ductal carcinomas positive for the breast [10]. HA is a ligand of the CD44 adhesion molecule. The cytosolic level of this proteoglycan can modulate certain clinical-biological properties in CD44 positive infiltrating ductal carcinomas of the breast [11,12].

In this context, HA has proved to be a significant ally in the development of nanotransporters that demonstrate preferential accumulation in tumors and increased cellular uptake. Since the discovery that the receptor is overexpressed in a variety of solid tumors, such as pancreatic, breast and lung cancer, many studies have focused on methods to target CD44, in an attempt to improve drug delivery and tissue discrimination. Healthy and malignant, while reducing residual toxicity and off-target accumulation [13].

In this sense, the expression of CD44 in breast tissues and its correlation with HA can generate an extensive accumulation of hyaluronan, serving as an additional prognostic factor. The hyaluronan in the tumor microenvironment promotes the proliferation of tumor cells, invasion, immune evasion, changes in stature, and drug resistance. In addition, possible clinical applications of targeting HA metabolism in cancer therapy are discussed [13].

Furthermore, the interaction of hyaluronan with CD44 receptors on the cell surface induces signaling events that promote cell growth independent of anchorage, survival, and migration, thus increasing metastatic dissemination. Increased HA synthesis in

cancer cells can lead to the formation of a less dense matrix that improves motility and invasion of tumor cells [2].

In addition, hyaluronan appears to form a coating that protects cancer cells from cytotoxic cells and chemotherapeutic agents. The level of HA showed an increase in highly metastatic breast carcinoma cells. In clinical samples of breast carcinoma, the expression of HA is regulated both in the cancer cells themselves and in the surrounding stroma and is an independent prognostic factor for patient survival (34). The presence of high levels of hyaluronan in myxoid stromal changes in breast cancer was strongly associated with a high degree of the tumor, tumor emboli with lymph node involvement, and increased mortality [2].

In this context, the combination of immunotherapy and chemotherapy is becoming a new and promising treatment for cancer. The big challenge is to target cancer and immune cells simultaneously and specifically. Thus, a dual system of multifunctional pH-responsive nanoparticles based on poly (L-histidine) and hyaluronic acid was designed to co-load R848 (immunoregulatory) and doxorubicin (chemotherapeutic) through different encapsulation modes. By responding to the acidic pH of the tumor microenvironment and intracellular organelles, this multifunctional nanoparticle system could release R848 extracellularly and provide DOX targeted to breast cancer cells, achieving synergistic effects of immunotherapy and chemotherapy against breast cancer [14].

4. State-of-the-Art of the Main Treatments

HA and CD44 regulate breast cancer cell phenotype and appear to predict the clinical outcome in patients with breast carcinoma. As the increase in HA, production leads to the resistance of cancer cells to various chemotherapeutic drugs, including doxorubicin, cisplatin, methotrexate, and paclitaxel [15]. The application of hyaluronidase to remove the immunoprotective and chemoprotective hyaluronan coating from cancer and reduce interstitial fluid pressure may improve the distribution of anticancer drugs and therapeutic antibodies against the tumor. In addition, the coupling of conventional chemotherapeutic agents with HA allows selective targeting of CD44 expression, thus decreasing the dosages of anticancer drugs administered, reducing undesired side effects [15].

Lipoplexes containing a hyaluronic acid-dioleoylphosphatidylethanolamine conjugate (HA-DOPE) were designed to target the CD44 receptor in breast cancer cells. Cationic liposomes composed of a mixture of [2- (2,3-didodecyloxypropyl) hydroxyethyl] ammonium (DE) and dioleoylphosphatidylethanolamine (DOPE) with or without HA-DOPE were prepared, characterized and used to form a complex with the DNA of the plasmid pCMV-Luc. The lipoplexes showed a negative zeta potential and an average diameter between 250-300 nm. Cytotoxicity was not modified by the presence of HA-DOPE. However, HA-DOPE increased the level of transfection in cells that express CD44 compared to the line that expresses very low levels of CD44. Thus, cationic liposomes containing the HA-DOPE conjugate mediated good transfection in cell lines that express CD44 in culture [16].

In addition, lapatinib (LPT) is a drug administered orally for the treatment of metastatic breast cancer [17]. To expand its therapeutic horizon, its nanocrystals (LPT-NCs) were prepared, which were later coated with hyaluronic acid (HA) to produce LPT-HA-NCs. Detailed in vitro and in vivo investigation of LPT-HA-NCs showed superior anticancer activity due to active targeting to CD44 receptors than the LPT-NCs and free LPT counterparts. In the triple-negative, 4T1 cells induced breast tumors with female Balb / C mice. Treatment with LPT-HA-NCs caused a significant delay in tumor growth and a general increase in the probability of survival of the animals due to their greater tumor location, increasing the residence time. The results suggested that the formulation of LPT-NCs coated with HA increases the activity of LPT against triple-negative breast cancer. It exhibited a magnificent therapeutic result at low doses, thus presenting a strategy to reduce dose administration and minimize dose-related toxicity [17].

An easy approach was developed to synthesize an innovative drug delivery platform for carbon nanoparticles with doxorubicin endowed with HA. The target carbon modified HA (CD-HA) CD44 points were synthesized as a carrier by hydrothermal treatment in one step within an hour with citric acid and branching PEI as a central carbon source [18]. HA not only functioned as a carbon point component but also as a target hydrophilic and ligand group in this system. The HA-CDs as prepared were then loaded with doxorubicin (HA-CD and p-CBA-DOX) through an acid cleavable bond, which released the drug in a pH-responsive manner. In in vitro experiments, HA-CD and p-CBA-DOX showed good hemocompatibility and serum stability, while exhibiting high cytotoxicity in

4T1 cells. The results of confocal laser scanning microscopy and flow cytometry demonstrated that DOX-loaded nanoparticles were internalized by 4T1 cells through the HA-mediated CD44 targeting effect. The increased tumor accumulation in vivo of HA-CD and p-CBA-DOX was witnessed by live imaging. Compared with free DOX, superior antitumor efficacy in vivo of HA-CD and p-CBA-DOX was observed in heterotopic and orthotopic 4T1 cell tumor models. In addition, hematological and biochemical analysis of blood demonstrated that HA-CD and p-CBA-DOX did not induce noticeable toxicity, which further confirmed the good biocompatibility of HA-CD and p-CBA-DOX. The formulated HA-CD and p-CBA-DOX provide an alternative strategy for therapy directed at breast cancer [18].

In this sense, the development of delivery systems capable of targeting tumors represents a promising strategy to overcome issues related to the non-specific effects of conventional anticancer therapies [19]. Currently, one of the most investigated agents for cancer targeting is HA, since its receptor, CD44, is overexpressed in many types of cancer. However, most studies on the CD44 / HA interaction have been performed so far in cell-free or genetically modified systems, thus leaving some uncertainty about which factors related to the cell influence the binding and internalization of HA in CD44- expressing cells. To address this, the expression of CD44 (standard and variants, designated CD44s and CD44v, respectively) was evaluated in human dermal fibroblasts (HDF) and in a large panel of cancer cell lines, including breast, prostate, head and neck, pancreas, ovarian, colorectal, thyroid, and endometrial cancer. The results showed that the CD44 isoform profiles and expression levels vary between cancer cell lines and HDF and are not consistent in cell origin. Using information composed of CD44 expression, HA binding, and internalization, it was found that CD44v expression can negatively influence HA uptake and, instead, when cells expressed mainly CD44s, a positive correlation was observed between expression and capture. The results show that factors other than the amount of CD44 receptor may play a role in the interaction with HA, and this represents an important advance with regard to the design of HA-based carriers and the selection of tumors to be treated according to the expression of CD44 [19].

In this context, metastasis is the main cause of cancer-related morbidity and mortality. The ability of cancer cells to become invasive and migratory contributes significantly to metastatic growth, which requires the identification of new anti-migratory and anti-invasive therapeutic approaches. Proteoglycan 4 (PRG4), a mucin-like glycoprotein, contributes to synovial homeostasis of the joints through its anti-adhesive and friction-reducing properties. Adherence to the components of the surrounding extracellular matrix (ECM) is essential for cancer cells to invade ECM and eventually become metastatic, raising the question of whether PRG4 has an anti-invasive effect on cancer cells [20].

Thus, one study reported that a full-length recombinant human PRG4 (rhPRG4) suppresses the ability of the secreted protein transforming growth factor-beta (TGFβ) to induce the phenotypic disruption of three-dimensional organoids derived from human breast cancer cells, reducing cells induced by invasion ligands. It was discovered that rhPRG4 suppresses TGFβ-induced invasiveness of cancer cells, inhibiting the cell surface cluster to hyaluronan (HA) downstream of the CD44 differentiation signal axis. In addition, rhPRG4 was found to suppress the TGFβ-dependent increase in the abundance of CD44 proteins and the enzyme HAS2, which is involved in HA biosynthesis. TGFβ has suppressive and tumor-promoting roles in cancer. Therefore, these findings point to the potential clinical utility of rhPRG4 as a therapeutic treatment for invasive and metastatic breast cancer [20].

5. Conclusion

The development of nanopharmaceuticals delivery systems is able to control the development of tumors and represent a promising strategy to overcome issues related to the non-specific effects of conventional anticancer therapies.

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Authors Contribution

Data collection, analysis and preparation of initial draft (CAG & CRA); Designing the study, data collection, analysis, preparation and finalising the manuscript (IJZF).

Data sharing statement

No additional data are available

Ethics Approval

Not Applicable

Informed consent

Informed written consent obtained from the participant

Conflict of interest

The authors declare no conflict of interest.

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