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Review

An overview of the effective combination therapies for the treatment of breast cancer

Cristina Núñez [a, c, *], José Luis Capelo [b, d], Gilberto Igrejas [c, e, f], Amparo Alfonso [a], Luis M. Botana [a], Carlos Lodeiro [b, d, **]

[a] Pharmacology Deparment, Faculty of Veterinary, University of Santiago de Compostela, 27002, Lugo, Spain
[b] BIOSCOPE Group, UCIBIO-REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516, Caparica, Portugal
[c] C4O Group, Research Unit UCIBIO-REQUIMTE, 2829-516, Caparica, Portugal
[d] ProteoMass Scientific Society, Madan Parque, Rua dos Inventores, 2825-182, Caparica, Portugal
[e] Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal
[f] Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

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ABSTRACT

Breast cancer (BC) is generally classified based on the receptors overexpressed on the cell nucleus, which include hormone receptors such as progesterone (PR) and estrogen (ER), and HER2. Triple-negative breast cancer (TNBC) is a type of cancer that lacks any of these three types of receptor proteins (ER/PR/HER2).

Tumor cells exhibit drug resistant phenotypes that decrease the efficacy of chemotherapeutic treatments. Generally, drug resistance has a genetic basis that is caused by an abnormal gene expression, nevertheless, there are several types of drug resistance: efflux pumps reducing the cellular concentration of the drug, alterations in membrane lipids that reduce cellular uptake, increased or altered drug targets, metabolic alteration of the drug, inhibition of apoptosis, repair of the damaged DNA, and alteration of the cell cycle checkpoints.

The use of "combination therapy" is recognized as an efficient solution to treat human diseases, in particular, breast cancer. In this review, we give examples of different nanocarriers used to co-deliver multiple therapeutics (chemotherapeutic agent and nucleic acid) to drug-resistant tumor cells, and lastly, we give our recommendations for the future directions for the co-delivery treatments.

1. Introduction

Breast cancer is the most common invasive malignancy in women with 450,000 annual deaths world-wide and the second leading cause of cancer-related death in women after lung cancer [1]. Breast cancer can be either in situ or invasive, with in situ cancers being relatively easily curable. It is invasive breast cancers, particularly invasive ductal carcinoma (80% of all invasive breast cancers) are a cause of great concern.

Breast cancer is clinically categorized on the basis of the existence of estrogen receptor (ER), the amplification of HER2/ErbB2 gene and the absence of three nuclear receptors, such as ER, progesterone receptor (PR) and HER2/ERBB2 (Triple Negative). While for the first two groups of breast cancer receptor-specific therapy is applied, chemotherapy remains the mainstay of treatment for triple negative breast cancer (TNBC) [2].

Some of the most common chemotherapeutic agent used for breast cancer treatment are Doxorubicin (DOX), Paclitaxel (PTX), Docetaxel (DCX), Thioridazine (THZ), Disulfiram (DSF), Camptothecin (CPT) and Curcumin.

Antibody-drug conjugates (ADCs) employed monoclonal antibodies (mAbs) to specifically bind tumor-associated target antigens and deliver a highly potent cytotoxic agent. Recently, ADCs have been used in cancer chemotherapy. The synergistic combination of mAbs conjugated to small-molecule chemotherapeutics, via a

* Corresponding author. Pharmacology Deparment, Faculty of Veterinary, University of Santiago de Compostela, 27002, Lugo, Spain.
** Corresponding author. BIOSCOPE Group, UCIBIO-REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516, Caparica, Portugal.
E-mail addresses: cristina.nunez@usc.es (C. Núñez), cle@fct.unl.pt (C. Lodeiro).

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stable linker, has given rise to an extremely potent class of anticancer drugs [3]. In particular, the more recent approval of trastuzumab emtansine (T-DM1), in 2014 [4], for use against breast cancer, proved that ADCs were adequate of targeting solid tumors in addition to haematological malignancies.

To overcome some limitations, various kinds of nanoparticles, like polymeric nanomicelles [5], polymer-drug conjugates [6], liposomes [7] and inorganic nanoparticles [8,9], have been developed as tumor-targeting vehicles for the selective delivery of these drugs.

Despite a significant progress in early diagnosis and treatment, resistance to conventional chemotherapeutic agents (i.e., chemoresistance) continuously poses a tremendous challenge to effective breast cancer therapy. In addition to metastasis, multidrug resistance (MDR) is a major obstacle in the treatment of breast cancer which may potentially lead to tumor relapse and the failure of therapy [10].

There are several forms of MDR: alterations in membrane lipids, efflux pumps, metabolic conversion of the drug, repair of the damaged DNA, inhibition of apoptosis, increased or modified drug targets, alteration of the cell cycle key points or alteration in microtubule-associated proteins (MAP) as cancer progress and mutation of β-tubulin [11,12]. In particular, the over-expression of ATP-binding cassette (ABC) transporters and the blocked apoptosis pathway, are two main origin of MDR [13].

P-glycoprotein (*P*-gp), one of the most well described ABC transporters, is over-expressed in the malignant tissues of almost 40–50% of breast cancer patients and becomes an attractive target to overcome MDR [14]. This *P*-gp encoded by the MDR-1 gene or Survivin, transports a variety of structurally and functionally diverse chemotherapeutic drugs thereby, reducing the accumulation of therapeutic concentration of drugs in MDR cancer cells, resulting in low cancer chemotherapeutic efficacy [15].

Several *P*-gp inhibitors have been explored over the last four decades to overcome MDR in cancer [16]. The first generation of ABC blockers such as verapamil [17], cyclosporine A [18], quinidine [19], promethazine [20] and Valspodar (PSC833) [21] are the most widely investigated probably because they are drugs already approved by the regulatory agencies for other uses [22] and easy to be clinically evaluated as ABC inhibitors for new intended uses. The nonspecific action of *P*-gp inhibitors on other molecular targets and/or their nonselectivity inducing intolerable toxicity that limited their usage in clinics [23].

Other MDR proteins such as MRP1, MRP2, BCRP and certain cell signaling pathways may contribute to chemoresistance.

In some cases, defects in the apoptotic networks of cancer cells greatly reduce the efficacy of chemotherapy drugs. A combination of agents that restore the apoptotic mechanism shows promise as an effective strategy for cancer treatment such as: expression of tumor suppressor genes like p53, retinoblastoma protein or MDA-7, introduction of apoptosis-inducing proteins like tumor necrosis factor-related apoptosis inducing ligand (TRAIL) or tumor necrosis factor-alpha (TNF-α). Other potential approaches rely on silencing of anti-apoptotic genes like sHRNA-expressing plasmid DNA targeting the survivin gene (shSur), Bcl-2 or BAX.

In order to understand the major contributory roles of different cellular mediators towards MDR development, novel small molecular drugs and biomacromolecules such as proteins, plasmid DNA, antisense oligonucleotides and siRNA to complement existing chemotherapeutic agents were used to inhibit the drug efflux pumps [24].

On this way, co-delivery strategy grows up to achieve the synergistic effect for cancer therapies and multiple factors need to be considered to select an optimal strategy: the need for sequential or simultaneous exposure, the kinetics of action of the individual agents and mutual chemical compatibility [25].

Compared to separate drug administration, co-delivery of multiple drugs in one system has several potential advantages, including: 1) design for better synergistic effects and avoid simply use of more drugs together, 2) greatly improve the patient compliance, and 3) accurately control the individual doses and avoid the uncertainty caused by dose fractionation [26].

In this development, the use of nanotechnology for the delivery of a combination of therapeutic agents confers many attractive attributes such as favorable biodistribution profiles, enhanced serum stability, controllable drug release, high carrier capacity, prolonged systemic circulation lifetime, reduced non-specific cellular uptake and multi-drug encapsulation for combinatorial treatment [27].

Moreover, nanocarrier drug delivery systems improve selectivity of anticancer drugs to tumor cells by enhanced permeability and retention (EPR) effect [28]. Inherently, drug-loaded nanoparticles also possess an edge against the drug efflux pumps of MDR in that the characteristic endocytic pathway for cellular internalization allows for the trafficking of the therapeutic agents away from the drug efflux mechanisms located on the cell membranes, hence permitting the intracellular accumulation and activity of the anticancer drug.

In this context, formulation the anticancer drugs in the various nanocarriers such as prodrugs, micelles, liposomes, solid lipid nanoparticles, nanoparticles of biodegradable polymers, nanohydrogels and dendrimers, have been developed and nanomedicine for multimodality treatment has been under intensive investigation [29]. Meanwhile, major efforts have been made to develop safer and less expensive nanocarriers [30].

During recent years, our research group has focused on the design of novel nanosystems [31–35] with biological [36] and biomedical applications [37–39], in particular, breast cancer treatment [40]. In this review, we collect recent progress on the development of different co-delivery nanocarrier systems and their applications in breast cancer therapy (Tables 1–3). We highlight the different novel codelivery material platforms and discuss the unique and vastly different set of challenges presented by the co-delivery of two fundamentally different therapeutic agents as compared to the conventional small drug molecules.

2. Simultaneous delivery: “co-delivery”

Recent reports have shown that co-delivery of two therapeutic agents presents synergistic effect and it could enhance the efficiency of delivering the two drugs to the same cell population by at least one order of magnitude when compared with delivering them in two separate carriers.

Therefore, co-delivery of two therapeutics in a single nanocarrier may offer benefits regarding convenience, synchronized pharmacokinetics, and delivery of defined, relative amounts of both agents to the same population of cells. Nevertheless, to optimize the overall anti-cancer activity and minimize the toxicity of combination treatments, it is necessary to understand the relationship among the mechanism of action of individual agents, the kinetics of their pharmacologic activity, and the mode of delivery. This improved understanding can, in turn, guide the design of co-delivery vector with appropriate timing and sequence of delivery of individual agents of the combination [41].

3. Materials used as carriers for co-delivery

Therapeutic treatments using therapeutic carrier has been researched for the past decade. These carriers can be made of