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Hyaluronic acid-chitosan nanoparticles for co-delivery of MiR-34a and doxorubicin in therapy against triple negative breast cancer

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ABSTRACT

Metastatic relapse, development of drug resistance in cancer cells and adverse side effects of chemotherapeutic agents are the major obstacles for effective chemotherapy against triple-negative breast cancer. To address these problems, miR-34a, a potent endogenous tumor suppressive molecule in breast cancer, was co-encapsulated with doxorubicin (DOX) into hyaluronic acid (HA)-chitosan (CS) nanoparticles (NPs) and simultaneously delivered into breast cancer cells for improved therapeutic effects of drug. DOX-miR-34a co-loaded HA-CS NPs were successfully prepared through ionotropic gelation method in water. *In vitro* and *in vivo* experiments showed that miR-34a and DOX can be efficiently encapsulated into HA-CS NPs and delivered into tumor cells or tumor tissues and enhance anti-tumor effects of DOX by suppressing the expression of non-pump resistance and anti-apoptosis proto-oncogene Bcl-2. In addition, intracellular restoration of miR-34a inhibited breast cancer cell migration via targeting Notch-1 signaling. The obtained data suggest that co-delivery of DOX and miR-34a could achieve synergistic effects on tumor suppression and nanosystem-based co-delivery of tumor suppressive miRNAs and chemotherapeutic agents may be a promising combined therapeutic strategy for enhanced anti-tumor therapy.

1. Introduction

Breast cancer is the most common invasive malignancy in women worldwide and the second leading cause of cancer-related death in women after lung cancer [1]. Despite a significant progress in early diagnosis and treatment, resistance to conventional chemotherapeutics continuously poses a tremendous challenge to effective breast cancer therapy. In addition to metastasis, drug resistance is a major obstacle in the treatment of breast cancer which may potentially lead to tumor relapse and the failure of therapy [2]. Therefore, strategies for the overcoming of drug resistance could provide rational therapeutic approaches to increase chemotherapy efficacy and improve the clinical outcome of breast cancer patients.

MicroRNAs (miRNAs) are a class of small, endogenous non-coding RNAs that post-transcriptionally control the translation and stability of mRNAs [3]. Dysregulation of miRNAs has been shown in many cancers and recognized as a hallmark of cancer. MiRNAs can function as tumor suppressors or on-miRs during tumor initiation and progression [4]. The expression of tumor suppressive miRNAs is usually lower in tumoral cells. Restoration of synthetic mimics of tumor suppressive miRNAs is designed to recover the suppressive function of the endogenous miRNAs and have shown to induce cell apoptosis and block the proliferation of cancer cells [5,6]. MiRNA-based anti-cancer therapies are thus being under development, either alone or in combination with conventional anti-cancer agents, such as chemotherapeutic drugs, with the goal of improving survival and clinical outcome [7]. One single miRNA can target multiple genes due to the imperfect complementarity with target mRNA and simultaneously regulate different signaling pathways, implying that miRNA may potentially play a major regulatory role in coordinating cancerous signaling networks [8]. Importantly, multiple-targeting ability of tumor suppressive

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miRNAs is displaying an increasing interest for improved tumor treatment due to the complexity of cancer-related signaling networks [9]. Therefore, the therapeutic approach to restore homeostasis by altering miRNA expression has a great potential to be a more practical strategy than silencing individual genes by siRNAs.

MicroRNA-34a (miR-34a) is a miRNA regulated by the p53 network at transcriptional level and has been shown to be remarkably downregulated in a variety of cancers [10]. Lower expression of miR-34a has been reported in triple negative and mesenchymal-type breast cancer cell lines. Exogenous expression of miR-34a in breast cancer cells induced cell apoptosis and inhibited cell proliferation and migration through targeting Bcl-2, CD44 and SIRT1 (silent information regulator 1), Rac1, Fra-1, Notch-1 and various cyclins [11–13].

A variety of viral carriers has been designed for miRNA delivery and has shown a high transfection efficiency over a broad range of cell types [14]. However, the safety concerns are currently perceived as hamper to the clinical application of viral vector-based therapy. Nanocarriers have been largely developed to encapsulate and deliver therapeutic agents, such as micelles and nanoparticles (NPs) formed with cationic polymers, polysaccharides, peptides and liposomes, and have attracted increasing attention in miRNA delivery due to their various advantages over the viral counterparts [15–18], including clinical potential and the ease of production. In addition to nanocarriers designed to deliver a single therapeutic agent, there are growing interests in developing multi-agent co-delivery nanocarriers that can simultaneously incorporate and deliver multiple types of therapeutic payloads to disease sites in a targeted and controlled manner for combined therapy [19–21]. These co-delivery nanocarriers may potentially allow to create synergetic effects of different therapeutic approaches to eventually improve overall treatment outcomes [22].

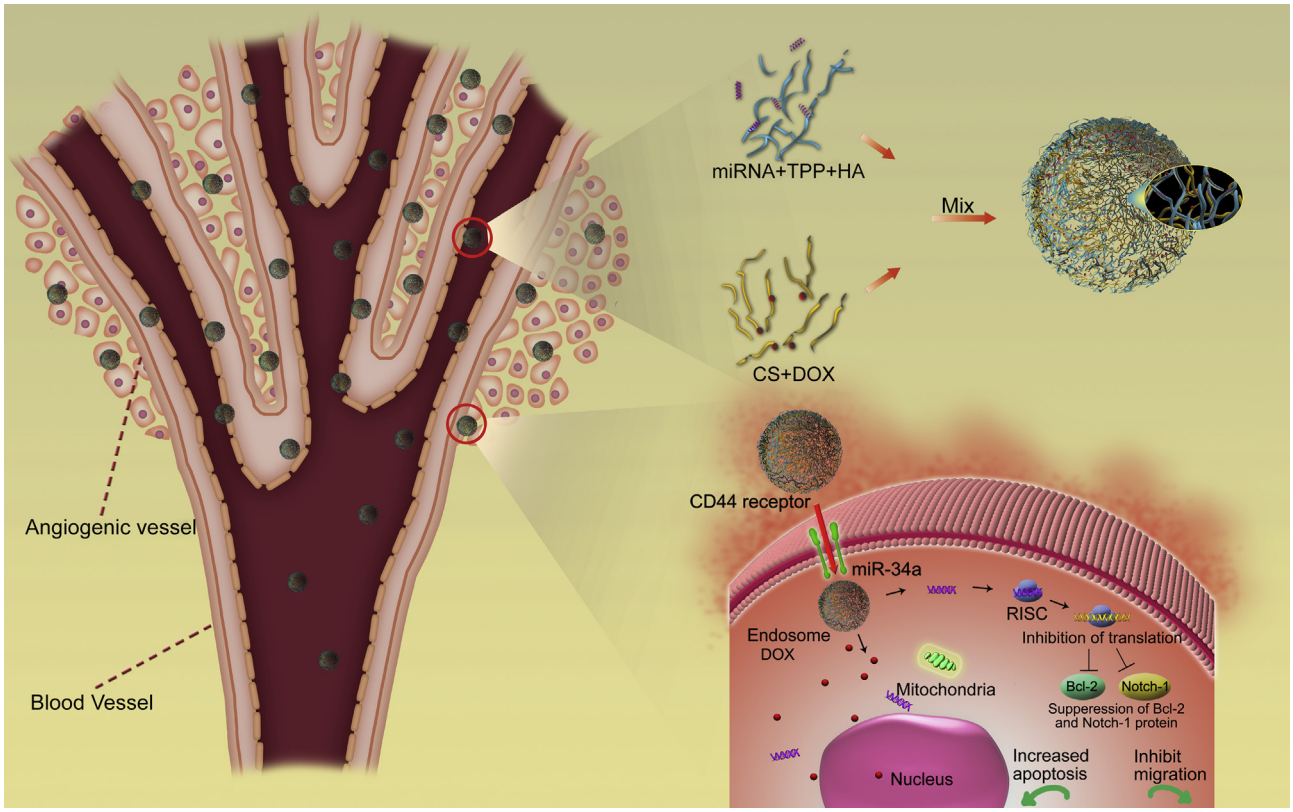
Nowadays, polyionic nano-complexes (PICMs), which are composed of polycations and opposite polyanions, have demonstrated a great potential in biomedical and nano-biotechnological applications [23], including the controlled drug release, good biocompatibility and gene transfection [24,25]. From the point of view of nano-complexes formulation, polyionic nanoparticles have the advantage of being highly versatile and secure as a nanosystem which can be easily adapted to different charged cargos. Among numerous polyionic nano-complexes systems, hyaluronic acid-chitosan nanoparticles (HA-CS NPs) have been broadly studied. Apart from its biocompatibility and biodegradability, the integrated HA backbone in itself is endowed with tumor-targeting property through specifically binding to CD44 molecule, an integral membrane glycoprotein over-expressed on the surface of various tumor cells, including MDA-MB-231 breast cancer cells [26], which makes it as an ideal polymer carrier for systemic drug delivery applications [27–29].

In our present studies, nano-complexes were prepared using hyaluronic acid and chitosan which simultaneously encapsulate positive charged Doxorubicin (DOX) and negative charged miR-34a mimics (Scheme 1). The particle size, surface zeta potential, morphology, DOX and miR-34a encapsulation efficiency, particle stability in various solutions and *in vitro* release were characterized. The synergetic effects and mechanisms of DOX and miR-34a in breast cancer therapy were further investigated by *in vitro* and *in vivo* models.

2. Materials and methods

2.1. Materials

Doxorubicin hydrochloride salt (DOX) was purchased from Beijing HuaFeng Co. LTD (Beijing, China). Cell counting kit-8 was purchased from Dojindo Molecular Technologies (Tokyo, Japan). MicroRNA-34a (MiR-34a) and FAM-labeled miR-34a



Scheme 1. Schematic illustration of the construction of HA-CS-based nanosystem for the simultaneous co-delivery of DOX and miR-34a to MDA-MB-231 human breast cancer cells for enhanced anti-cancer effects.