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Targeting epithelial-mesenchymal transition: Metal organic network nano-complexes for preventing tumor metastasis

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ABSTRACT

Tumor metastasis is the leading cause of death in cancer patients, and epithelial-mesenchymal transition (EMT) is an essential step in tumor metastasis. Unfortunately, during the chemotherapy, EMT could be induced under the selective pressure of clinical cytotoxic drugs. Here, to solve this problem, we have synthesized multi-functional epigallocatechin gallate/iron nano-complexes (EIN) as a versatile coating material to improve conventional therapies. *In vitro* studies showed that this strategy could eliminate EMT-type cancer cells. Mechanism studies also revealed that EIN was able to down-regulate the downstream expression of metastasis-associated factors, decrease the migration ability of cancer cells and prevent cancer cells from gaining drug resistance. *In vivo* investigation revealed that EIN had superior ability to enhance the therapeutic effect of conventional nanomedicines and inhibit the EMT process. Our study indicates the promising use of EIN to make up for the deficiencies of chemotherapy may provide insights into systematic cancer therapy to overcome tumor metastasis and drug resistance.

1. Introduction

Tumor metastasis is the leading cause of the high mortality of cancers with poor prognosis [1]. Despite recent advances in tumor treatments, therapeutic methods for tumor metastasis treatments are still very limited. Recently, a range of strategies have been proposed to fight against tumor metastasis. Among them, designing metastatic tumor targeting materials is a promising method to treat advanced cancers. However, this strategy is restricted by limited differences between metastatic tumors and primary tumors [2,3]. In earlier studies, researchers mainly focused on the capture of circulating tumor cells. Nevertheless, to eliminate all circulating tumor cells in the whole circulatory system remains a great challenge and few escaped circulating tumor cells could cause serious distant metastasis [4,5]. To date, increasing evidences have suggested that tumor microenvironment (TME) normalization strategies such as tumor oxygenation, acidic environment neutralization and drug resistance reversion are effective solutions for metastasis prevention. Besides, various nanomaterials such as g-C_3N_4 nanoparticles, MnO_2 nano-sheets and metal-organic frameworks have been used to alleviate tumor hypoxia and neutralized acidic environment [6–9]. Therefore, an ideal material for effective metastasis prevention should have satisfactory performance in TME normalization [10,11].

As it is well known, with the effect of chemotherapeutic agents, cancer cells would undergo adaptive changes and update their drug resistance ability, which make chemotherapeutic agents less effective [12]. Emerging evidences also demonstrate that the development of drug resistance could induce specific variations, which are consistent with EMT, a dominant step involves in tumor metastasis [13–15]. Furthermore, cancer cells which undergo EMT, would up-regulate the expression of metastatic factors including matrix metalloproteinase (MMP) and vascular endothelial growth factor (VEGF) to induce extracellular matrix degradation and trigger neovessel formation [16]. These changes could provide escape routes for cancer cells to leave the primary tumor and spread to other organs such as lung and liver [17]. As a consequence, to eliminate EMT type cancer cells that with both

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intensified drug resistance and high metastatic capability is of great importance during chemotherapy.

Traditionally, small molecule inhibitors were used to overcome drug resistance. However, the non-specific inhibition of P-glycoprotein (P-gp) would frequently increase side effects of chemotherapeutics due to the compromised drug elimination in liver and kidney. Nano-carries have been demonstrated to enhance the therapeutic effect of small molecule agents by improving the tumor targeting capability and reducing the side effect *via* their enhanced permeability and retention (EPR) effect [18–20]. Thus, designing a nanomedicine to inhibit the development of drug resistance within primary tumor would be a superior way to overcome the shortcoming of traditional inhibitors.

Tea polyphenols are a series of compounds found in tea. A FDA approved tea polyphenol, epigallocatechin gallate (EGCG), is found to surmount drug resistance through modulating of cell signaling and finally down-regulate the expression of P-gp [21,22]. Previous studies show that catechol containing molecules could assemble with metal ions to prepare versatile films for nanoparticles coating [23–26]. Keeping all these issues in mind, in this work, we constructed a functional coating membrane through coordinating epigallocatechin gallate (EGCG) with ferric ions to prepare epigallocatechin gallate/iron nano-complexes (EIN) with ideal biocompatibility and low cytotoxicity.

Furthermore, as a universal coating material, EIN was used to coat traditional drug delivery nanoparticles such as mesoporous silica nanoparticles (MSN) and PEG-PLA micelles (Mic) to form MSN@EIN and Mic@EIN, respectively. Inherited from EGCG, MSN@EIN and Mic@EIN showed a high capability to enhance the intracellular drug concentration and to prevent drug resistance during chemotherapy. According to previously studies, although the combined administration of free EGCG and free doxorubicin (DOX) could also prevent cancer cells from gaining drug resistance, significant toxicity to liver and kidney could not be neglected as revealed by blood biochemical analysis. Herein, DOX@MSN@EIN and DOX@Mic@EIN drug delivery systems were prepared to avoid the shortcoming of small molecule inhibitors with minimized liver and kidney toxicity. In the follow up study, we disclosed that EIN also eliminated EMT-type cells and restricted the tumor metastasis *in vitro* and *in vivo*. Detailed mechanism studies demonstrated that the presence of MSN@EIN and Mic@EIN in the drug delivery systems notably inhibited drug resistance and eliminated EMT-type cells. Besides, the inhibited EMT process of cancer cells further down-regulated the expression of metastasis factors such as matrix metalloproteinases (MMP) including MMP-2/9 and VEGF. Overall, EIN encapsulation is a versatile and universal method for upgrading current nanomedicines, which provides a novel strategy to develop anti-cancer agents.

2. Materials and methods

2.1. Materials

Methanol, dimethyl formamide (DMF), sodium hydroxide (NaOH), triethylamine (TEA), dimethyl sulfoxide (DMSO), dichloromethane, hydrochloric acid (HCl), ethylsilicate (TEOS), hexadecyl trimethyl ammonium bromide (CTAB), and hydrofluoric acid were purchased from Shanghai Reagent Chemical Co. H_2O_2, tris-(hydroxymethyl) aminomethane (Tris), (−)-epigallocatechin-3-gallate (EGCG) and ferric (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) were from Aladdin Industrial Corporation. Ferrous lacate (edible grade) was purchased from Zhengzhou Hongxiang Chemical Co. Ethylene diamine tetraacetic acid (EDTA), Tween 20, catalase and urea were supplied by Sigma-Aldrich. PEG_{2000}-PLA_{2000} was obtained from Xian Ruixi Biological Technology Co., Ltd. Toluene, DMF and TEA were redistilled before used. Doxorubicin hydrochloride (DOX) was provided by Zhejiang Hisun Pharmaceutical Co. (China). Antibodies were obtained from Abcam and Bioss.

2.2. Preparation of DOX@MSN

1.0 g of CTAB (274 mM) and 280 mg of NaOH (7 mM) were dissolved in 480 mL of DI water and kept at 80 °C for 0.5 h. Then 5.0 g of TEOS (24 mM) was added dropwise for 20 min under vigorous stirring. After 2 h, the resulting product was centrifuged (10,000 r/min, 15 min), and washed with DI water and methanol for three times. Then, the mixture of 200 mg of CTAB@MSN, 80 mL of methanol and 5 mL of HCl was refluxed at 80 °C for 48 h. The resulting product was centrifuged (10,000 rpm, 15 min), and washed with methanol and water several times. After that, 100 mg of MSN was suspended in a methanol/H_2O mixture with 20 mg of DOX (36.8 μM) and stirred for 24 h. The product was washed with methanol and water, and then lyophilized for 3 days.

2.3. Preparation of DOX@Mic

5 mg DOX (9.2 μM) (desalt) and 50 mg PEG-PLA were dissolved in 2 mL dichloromethane in a round-bottom flask. The solvent was evaporated by rotary evaporation at room temperature under vacuum to obtain DOX@Mic. The product was dissolved in DI water and dialyzed for 2 days.

2.4. Preparation of DOX@MSN@EIN and DOX@Mic@EIN

5 μL of polyphenol solution (1 mM) and 5 μL of $FeCl_3.6H_2O$ (1 mM) solution were added to 500 μL of DOX@MSN or DOX@Mic containing solution (0.1 mg/mL), respectively. The mixture was vigorously mixed under ultrasonication for 30 s immediately after the individual additions of polyphenol and $FeCl_3 \cdot 6H_2O$. The pH value of this mixture was then raised to 8.0 by adding 0.5 mL of tris buffer (20 mM, pH 8.5). The product was dialyzed against water for 2 days to obtain EIN coated nanoparticles.

2.5. Cell culture and in vitro toxicity

The cell toxicity was examined by MTT assay. 4T1 cells was seeded in a 96-well plate with a cell density of 5×10^4 per well, and then incubated in 100 μL of cell culture medium containing 10% FBS in an incubator (37 °C, 5% CO_2) for 24 h. The medium containing a particular agent was added to each well respectively. After 48 h, the cells were washed with EDTA to remove the Fe^{3+}. After that, the medium was replaced with 200 μL of fresh medium and 20 μL of MTT (5 mg/mL in PBS buffer) was added. After incubation at 37 °C for 4 h, the medium was removed and 150 μL of DMSO was added. The absorbance was measured at 570 nm using a microplate reader (Bio-Rad, Model 550, USA). The relative cell viability was calculated by ($OD_{570sample}/OD_{570control}$) × 100%, where $OD_{570control}$ was obtained in the absence of the agent and $OD_{570sample}$ and in the presence of the agent.

2.6. Cellular uptake

Cells were seeded in a small plate with a density of 1×10^5, and incubated with 1 mL of medium containing 10% FBS at 37 °C for 24 h. 1 mL of medium containing a particular agent was added. After incubated with cells for 4 h, the medium was removed and cells were washed three times. Then cells were observed with CLSM. (Nikon C1-si, TE2000, Japan).