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Treatment of cancer micrometastasis using a multicomponent chain-like nanoparticle

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ABSTRACT

While potent cytotoxic agents are available to oncologists, the clinical utility of these agents is limited due to their non-specific distribution in the body and toxicity to normal tissues leading to use of suboptimal doses for eradication of metastatic disease. Furthermore, treatment of micrometastases is impeded by several biobarriers, including their small size and high dispersion to organs, making them nearly inaccessible to drugs. To circumvent these limitations in treating metastatic disease, we developed a multicomponent, flexible chain-like nanoparticle (termed nanochain) that possesses a unique ability to gain access to and be deposited at micrometastatic sites. Moreover, coupling nanochain particles to radiofrequency (RF)-triggered cargo delivery facilitated widespread delivery of drug into hard-to-reach cancer cells. Collectively, these features synergistically facilitate effective treatment and ultimately eradication of micrometastatic disease using a low dose of a cytotoxic drug.

1. Introduction

The vast majority of breast cancer mortality is due to metastatic disease [1]. For example, the 5-year survival rate of breast cancer patients sharply decreases from 98% in cases with localized primary lesions to 23% in cases of distant metastases [1]. Although oncologists have potent small molecule chemotherapeutics such as anthracyclines (*e.g.* doxorubicin; abbreviated as DOX), the dose of these agents is constrained by their toxicity to normal tissue, because they are distributed within cancer and healthy tissues in a non-specific manner [2].

Due to the unique structures and material properties that appear at the nano-scale, nanoparticles provide new opportunities to address the complexity of cancer metastasis. To date, though, applications of nanotechnology have mainly focused on primary tumors. Nanoparticles have been developed to exploit the leaky vasculature of primary tumors to enhance the intratumoral drug delivery due to the so-called 'Enhanced Permeability and Retention' (EPR) effect [3–7]. While the EPR strategy may be effective in well-vascularized tumors larger than 100 mm^3, it is ineffective in micrometastatic disease, which presents small clusters of malignant cells within variable tissue types [8,9]. However, targeting an occult lesion hidden within a large population of normal cells presents a unique challenge [8].

To overcome these limitations of current drugs in their molecular or nanoparticle form, we designed a multicomponent nanochain (termed nChain), which is comprised of three iron oxide (IO) nanospheres and one drug-loaded liposome chemically linked into a linear, chain-like assembly (Fig. 1a,b). The multicomponent nature and shape of the nChain particle result in two unique features that facilitate enhanced treatment of difficult-to-reach cancer sites. The micrometastasis-specific features of the nChain-based therapy are illustrated in Fig. 1c. First, the nChain particle is capable of transporting a large drug cargo to metastases *via* vascular targeting of the endothelium associated with micrometastasis. The nChain utilizes a cyclic RGD peptide as a ligand to target the $\alpha_v\beta_3$ integrin receptor, which has a well-established role in the development of breast cancer metastasis [10–16]. While initial adhesion of circulating tumor cells onto the endothelium involves cell rolling, the metastatic site quickly transitions to firm attachment that is mediated by $\alpha_v\beta_3$ integrin [10,12–15]. Although $\alpha_v\beta_3$ integrin mediates the adhesion of cells to a large number of extracellular matrix proteins, it is minimally

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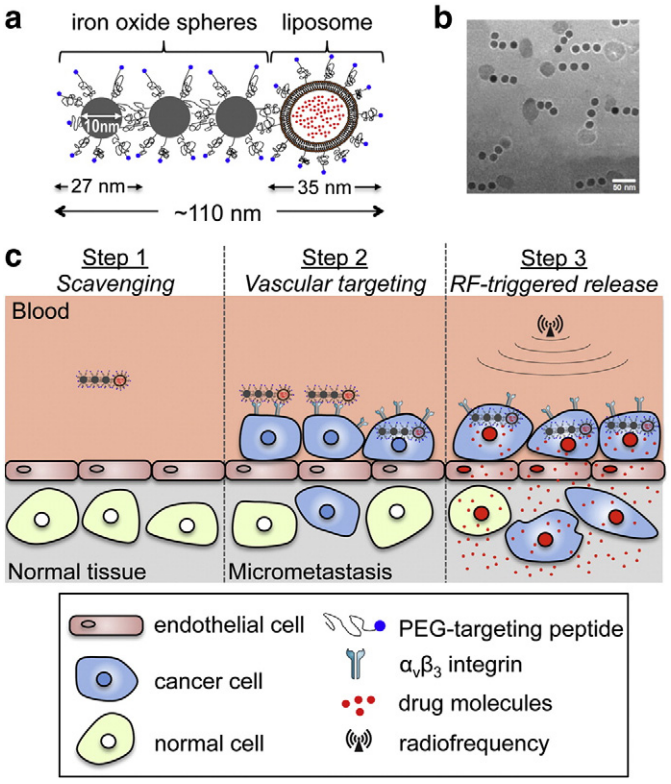

Fig. 1. Illustration of the nChain particle and its therapeutic effect on micrometastasis. (a) Schematic of a linear nChain particle composed of three IO nanospheres and one drug-loaded liposome. (b) TEM image of nChain particles. (c) Illustration of the successful delivery of nanochain-based drug to metastasis *via* vascular targeting and RF-triggered drug release.

expressed on normal blood vessels [17–19]. Thus, $\alpha_v\beta_3$ integrin-mediated vascular targeting can be highly specific towards blood vessels associated with metastatic disease, particularly since extravascular metastases are preceded by metastatic cancer cells residing inside the lumen of blood vessels [10,20,21]. Furthermore, the size, shape and flexibility of nChain particles substantially increase their probability of homing to micrometastases. The structure of the nChain particle increases both the lateral drift and margination of nanoparticles towards the blood vessel walls in microcirculation (*i.e.* continuous scavenging of vascular walls), and targeting avidity of nanoparticles (*i.e.* latching on vascular target) due to geometrically enhanced multivalent attachment on the vascular target [22].

However, even after successful targeting of a nanoparticle to micrometastasis, the overall effectiveness of this event primarily reflects the biological activity of "free" drug against neighboring cancer cells. While nanoparticles typically release their content slowly, drug release from nChain particles can be remotely triggered due to mechanically induced defects of the liposomal membrane caused by the oscillation of the IO portion of the nChain in the presence of a mild radiofrequency (RF) field [19]. For these analyses, we chose the drug DOX, which can rapidly diffuse through cellular membranes and reach nuclear DNA, which functions as a sink for DOX [23–25]. Application of an RF field rapidly liberated DOX molecules from nChain particles resulting in widespread anticancer activity throughout micrometastatic sites.

2. Materials and methods

2.1. Materials

The 4T1-GFP-luc cell line was received as a gift from Dr. Ruth Keri (Case Western Reserve University, Cleveland, OH). Female Balb/c mice were purchased from Charles Rivers (Wilmington, MA). The primary antibody for the specific endothelial antigen CD31 was purchased from BD Biosciences Pharmingen (San Diego, CA). Secondary antibodies and cell culture media were obtained from Invitrogen (Carlsbad, CA). The TUNEL assay kit was purchased from Roche Diagnostics (Indianapolis, IN). Cross-Linked Ethoxylate Acrylate Resin (CLEAR) resin, reaction vessels, other accessories for solid-phase chemistry and the cyclo (Arg-Gly-Asp-D-Phe-Cys) or c(RGDfC) peptide were purchased from Peptides International Inc. (Louisville, KY). The crosslinkers 3,3′-Dithiobis(sulfosuccinimidylpropionate) (DTSSP) and sulfosuccinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate (sulfo-SMCC), and the cleaving agent Tris[2-carboxyethyl] phosphine (TCEP) were obtained from Thermo Fisher Scientific (Cleveland, OH). Polyethylene glycol (PEG) conjugates were purchased from Laysan Bio (Arab, AL). General solvents and chemicals were obtained from Thermo Fisher Scientific (Cleveland, OH). Doxorubicin (DOX) was obtained from Sigma (Saint Louis, MO).

2.2. Synthesis and characterization of nanoparticles

To fabricate the multicomponent nChain particles, we employed a stepwise solid-phase chemistry approach to assemble the particles following a modification of a previously published method [19,26]. In the first step, solid-phase chemistry was used to partially modify the surface functionality of IO nanospheres with a hydrodynamic diameter of 27 nm (the size of the IO core is 10 nm) (Fig. 2a). Amine-PEG-functionalized IO nanospheres were conjugated onto amine-functionalized CLEAR resin *via* a homobifunctional crosslinker (DTSSP) reactive towards amines containing a cleavable disulfide bridge. The IO nanospheres were allowed to bind to the solid support and then cleaved off using a reducing agent (TCEP). The thiolytic cleavage liberated the IO nanosphere from the solid support converting the amines to a different chemical functionality (thiol group) on the portion of the nanosphere's surface that was linked to the resin. In the second step, by defining the topology of two different functional groups on the surface of the parent nanospheres, the two unique faces on the parent IO nanosphere served as fittings to chemically assemble them into nanochains using solid-phase chemistry (Fig. 2b). The same type of resin was used and the modified nanospheres were introduced in a

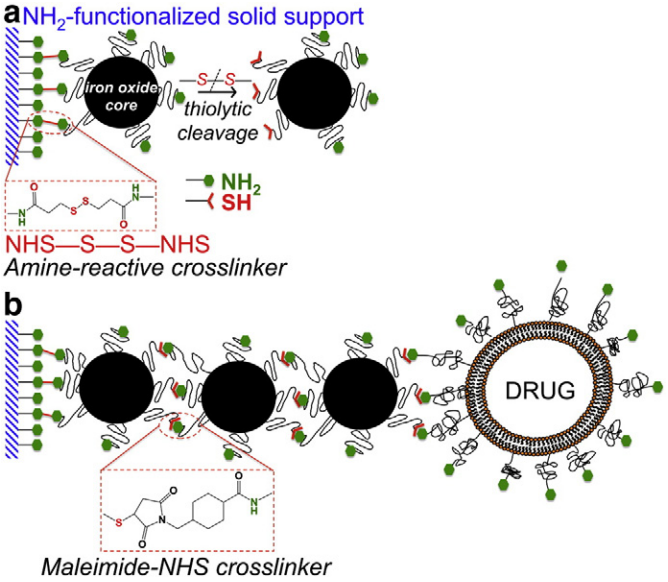

Fig. 2. Reaction scheme of the controlled assembly of multicomponent nChain particles using solid-phase chemistry. (a) In the first step, chemical bifunctionality on the surface of parent IO nanospheres is topologically controlled resulting in nanospheres with two faces, one displaying only amines and the other only thiols. (b) In the second step, the two unique faces on the parent nanosphere serve as fittings to chemically assemble them into nanochains.