Biomaterials 137 (2017) 23–36

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Treating metastatic triple negative breast cancer with CD44/neuropilin dual molecular targets of multifunctional nanoparticles

De-Sheng Liang [a, b], Wen-Jie Zhang [a], Ai-Ting Wang [a], Hai-Tao Su [a], Hai-Jun Zhong [b], Xian-Rong Qi [a, *]

[a] Beijing Key Laboratory of Molecular Pharmaceutics and New Drug Delivery System, School of Pharmaceutical Sciences, Peking University, 38 Xueyuan Road, Haidian District, Beijing 100191, PR China
[b] School of Pharmacy, Nanchang University, 461 Bayi Road, Donghu District, Nanchang 330006, PR China

ARTICLE INFO

Article history:
Received 6 March 2017
Received in revised form
12 May 2017
Accepted 12 May 2017
Available online 13 May 2017

Keywords:
Cancer metastasis
Neuropilins
CD44 receptor
Tumor neovasculature
Targeting delivery
Triple negative breast cancer

ABSTRACT

Metastasis of cancer makes up the vast majority of cancer-related deaths, and it usually initiates from tumor cells invasiveness and develops through tumor neovasculature. In this work, we have fabricated a CD44/neuropilin dual receptor-targeting nanoparticulate system (tLyP-1-HT NPs) with endogenous or FDA approved components for treating metastatic triple negative breast cancer (TNBC). The enhanced specific targeting of tLyP-1-HT NPs to both metastatic tumor cells and metastasis-supporting tumor neovasculature was contributed by means of CD44/neuropilin dual receptor-mediated interaction. The NPs not only effectively suppress the invasive capability of tumor cells themselves, but also significantly restrain the metastasis incidence via extravasation as well as the eventual colonization in lungs. In all the three types of TNBC-bearing mice models, orthotopic, post-metastasis and metastasis prevention models, the docetaxel-loaded tLyP-1-HT NPs exhibited markedly enhanced anti-tumor and anti-metastasis efficacy. The inhibitory rates of tLyP-1-HT NPs against orthotopic tumor growth and lung metastasis achieved 79.6% and 100%, respectively. The metastasis inhibition rate and life extension rate of the tLyP-1-HT NPs against post-pulmonary metastasis mice reached 85.1% and up to 62.5%, respectively. All the results demonstrated the designed dual receptor-targeting multifunctional NPs hold great potential in treating metastatic TNBC and lung metastasis.

1. Introduction

Cancer metastasis causes the vast majority of cancer deaths. For many patients, metastasis has already occurred when cancer is detected. The unique challenges for treating metastases include their small size, high multiplicity and dispersion to diverse organ environments. Breast cancer is the most prevalent malignancies among women worldwide [1]. As tumor metastasis occurs, the traditional surgical operation, radiotherapy or chemotherapy become much less successful [2,3]. In fact, 90% of breast cancer-related deaths for patients result from tumor metastasis. The 5-year survival rate for advanced or metastasized breast cancer is only 26% and the complete therapeutic response for patients is almost rarely achieved [4]. Triple negative breast cancer (TNBC) as one of breast cancer subtypes exhibits the worst survival rate due to its highly aggressive and metastatic behavior. Data from clinical reports indicate that the median overall survival (OS) for patients with metastatic TNBC (9–13.3 months) is much shorter than that for patients with other metastatic breast cancer subtypes (28 months) [5,6]. Therefore, developing an effective theranostic delivery platform for TNBC remains to be an urgent medical need.

Metastasis is a multi-stage process that generally involves local invasion and migration of tumor cells from primary sites, intravasation into adjacent vasculature, transit through circulation, extravasation into the distant organs and eventual colonization and formation of micro-metastases [7,8]. An early event that is essential for tumor cell metastasis is loss of intercellular junctions and acquisition of motile and invasive property via the epithelial-to-mesenchymal transition (EMT) process through cooperatively activating multiple signaling pathways or functional proteins [9–11]. Among them, CD44 receptor, a widely expressed adhesion molecule, has been actively implicated in cancer cell dissemination in early stage by regulating cell-cell and cell-matrix adhesion, cell

* Corresponding author.
E-mail address: qixr@bjmu.edu.cn (X.-R. Qi).

http://dx.doi.org/10.1016/j.biomaterials.2017.05.022
0142-9612/

proliferation, migration, growth, differentiation and trafficking in cooperation with other cellular functional proteins [12,13]. Elevated CD44 expression level has clinically demonstrated to be positively correlated with the high invasiveness and poor prognosis of cancer [14,15]. Furthermore, CD44 has also proved to be of great significance in maintaining stem cell phenotype and as one of the most common cell surface biomarkers for stem cells of several types of highly recurrent cancer, which consists of a minor fraction of all tumor cells, but innate drivers of tumorigenesis and metastasis [16,17]. Namely, all these findings suggest that CD44 receptor plays an important role in the development of tumor metastasis, which is potentially a theranostic target in treating metastatic cancers.

Over the past decades, considerable research attention has been given to the use of nanotechnology and nanomedicine to combat against cancer metastasis [18,19]. The nanomedicines are eligible to deliver the therapeutic agents efficiently with features of improved pharmacokinetics, targeted delivery, and reduced side-effects [20–22]. The nanomedicines less than 200 nm are easily accumulated in tumor regions and stay longer due to enhanced permeability and retention (EPR) effect in highly-fenestrated tumor endothelial cells, but poor permeation in normal vasculature [23]. On the basis of passive targeting delivery via EPR effect, active targeting strategies for introducing tumor molecularly targeted moieties (i.e. ligands, peptides, antibodies) to nanomedicines have also been investigated. The special interaction of ligand-conjugated nanomedicines with tumor cells surface receptors is thought to favor effectiveness of drug delivery with high selectivity [24,25]. It is demonstrated that the well-studied molecular markers associated with tumor cell motility and invasion, including integrins, CD44, EGF receptors and so on are all viable targets for achievement of metastatic cancer-targeted delivery [26–28]. As far as CD44 expressing cancer cell-targeting is concerned, the most commonly applied approach is centered on hyaluronic acid (HA), which is a natural glycosaminoglycan and intrinsic ligand for CD44 [29]. It is noteworthy that molecular weight (MW) of HA affects its biological functions. High MW HA is considered to be anti-angiogenic and anti-inflammatory, while low MW HA appears to be pro-angiogenic and pro-inflammatory [30]. However, several previous researches have demonstrated that low MW HA was functionalized to a variety of nanoparticles (NPs) with high safety profiles to efficiently target the metastatic tumor and to enhance *in vivo* therapeutic efficacy [31–35].

However, the singly tumor cell-oriented targeting strategy is inherently not qualified to cure metastatic cancer, due in significant part to the complex crosstalk in tumor microenvironments that contrive tumor cell metastasis. One typical event is initiation of neovasculature to offer available spreading routes for tumor cells into secondary site [36,37]. Indeed, high tumoral neovascularization has been demonstrated to be a prognostic indicator in many invasive cancers [38,39]. It appears that the neogenesis of tumor vascularity allows for enough lumen space for tumor cells upon intravasation and sufficient rate of blood flow to carry away the intravasated cells from primary site to next, increasing metastasis incidence [40]. It has been shown that targeting to restrain tumor neovasculature could significantly suppress the tumor cells proliferation and metastasis [41]. Hence, it is favorable to exploit the improved targeting strategy to both metastatic tumor cells and metastasis-supporting tumor neovasculature for metastatic cancer therapy.

Neuropilins (NRPs), the modular transmembrane proteins, serve as coreceptors for several members of the vascular endothelial growth factor (VEGF) family for regulating tumor cells growth and tumor vasculature sprouting [42]. Both members of neuropilin-1 (NRP-1) and its homologue neuropilin-2 (NRP-2) are overexpressed in several types of tumor cells, as well as widely present in tumor endothelial cells, where NRP-1 is closely involved in angiogenesis and NRP-2 in lymphangiogenesis [43–46]. Of great interest to us, the finding that NRPs bind and internalize the exogenous substrates (e.g. tLyP-1 peptide), so-called tumor-specific CendR peptides, can be availed for the design of improved targeting nanovehicles to both metastatic tumor cells and metastasis-supporting neovasculature [47].

Here we developed the multifunctional NPs with CD44/neuropilin dual receptor targeting (tLyP-1-HA NPs) for highly metastatic TNBC therapy. The NPs were constructed through molecular assembly of two modularized amphiphilic conjugates, one was low MW hyaluronic acid (LMW-HA, 7.8 kDa) and D-α-tocopheryl succinate (α-TOS) conjugates (HT), it was expected that the LMW-HA-based conjugate possessed the ability to accumulate and penetrate in highly metastatic tumors by CD44-mediated interaction [31]; the other was tLyP-1-poly(ethyleneglycol)2000-TOS (tLPTS), it was expected that the tLyP-1 targeting moiety modification possessed the enhanced specificity to highly metastatic tumors as well as to metastasis-supporting neovasculature by neuropilin-mediated interaction [47]. Additionally, α-TOS and TPGS in the multifunctional nanoparticles could serve, respectively, to activate mitochondrial apoptotic pathways inside tumor cells, and as an inhibitor of P-gp to overcome the multidrug resistance (MDR). Thus, it is hypothesized that targeting to both metastatic tumor and tumor neovasculature by the multifunctional NPs is favorable for improved anti-metastatic cancer therapy. The NPs hold the size around 120 nm and have high propensity for long blood circulation and enhanced tumoral accumulation through the EPR and CD44/neuropilin dual receptor-mediated interaction. Our results showed that tLyP-1-HT NPs significantly inhibited the growth of TNBC and the incidence of metastasis by enhancing the targeting delivery specificity to both metastatic tumor and metastasis-supporting neovasculature, as well as to the metastatic lung foci in several orthotopic and lung metastasis mouse models.

2. Materials and methods

2.1. Materials

Hyaluronic acid (HA, 7.8 kDa) was provided by Shandong Freda Biochem Co., Ltd. (Shandong, China). tLyP-1 peptide (CGNKRTR, MW 833.97) was synthesized by GL Biochem. (Shanghai, China). Docetaxel (DTX) and Taxotere® were commercially available from Norzer Pharmaceutical Co., Ltd. and the local hospital of Beijing, respectively. Coumarin-6 (COU) and DiR fluorescent probes were provided by Life Technologies (Eugene, OR, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Sigma-Aldrich Co., Ltd. (St. Louis, MO, USA). Lyso-Tracker Red and ER-Tracker Red were purchased from Invitrogen (Carlsbad, CA, USA) and Beyotime Biotech. Co., Ltd. (Jiangsu, China), respectively. Matrigel was obtained from BD Biocoat (Franklin, NJ, USA). Crystal violet was provided by Amresco (Solon, OH, USA). Rabbit polyclonal to CD31 antibody and rabbit polyclonal to LYVE-1 antibody were purchased from Abcam (Cambridge, MA, USA). FITC-labeled secondary antibody was obtained from Proteintech (Chicago, IL, USA). RPMI 1640 medium, Leibovitz's L15 medium, DMEM medium, penicillin-streptomycin, trypsin, 4% formaldehyde and Hoechst 33258 were provided by Macgene Co., Ltd. (Beijing, China). Fetal bovine serum (FBS) was obtained from Invitrogen/Gibco (Grand Island, NY, USA). Bouin's solution was provided by Leagene Biotech. Co., Ltd. (Beijing, China). D-luciferin potassium salt was obtained from Gold Biotech. Inc. (St. Louis, MO, USA). All other reagents and chemicals were of analytic grade and from commercial sources.

The female BALB/c mice and BALB/c nude mice (16–18 g) were