Journal of Controlled Release 217 (2015) 27–41

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Nanoparticle modulation of the tumor microenvironment enhances therapeutic efficacy of cisplatin

Lei Miao [a], Yuhua Wang [a], C. Michael Lin [a], Yang Xiong [a,b], Naihan Chen [a], Lu Zhang [a], William Y. Kim [c], Leaf Huang [a,*]

[a] Division of Molecular Pharmaceutics and Center of Nanotechnology in Drug Delivery, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
[b] School of Pharmacy, Zhejiang Chinese Medical University, 310053, China
[c] Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

ARTICLE INFO

Article history:
Received 19 March 2015
Received in revised form 1 August 2015
Accepted 13 August 2015
Available online 15 August 2015

Keywords:
Nanoparticle
Wnt16
Tumor microenvironment
Cisplatin
Tumor associated fibroblast

ABSTRACT

The tumor microenvironment (TME) serves as a multidrug resistant center for tumors under the assault of chemotherapy and a physiological barrier against the penetration of therapeutic nanoparticles (NPs). Previous studies have indicated the ability for therapeutic NP to distribute into, and deplete tumor-associated fibroblasts (TAFs) for improved therapeutic outcomes. However, a drug resistant phenotype gradually arises after repeated doses of chemotherapeutic NP. Herein, the acquisition of drug resistant phenotypes in the TME after repeated cisplatin NP treatment was examined. Particularly, this study was aimed at investigating the effects of NP damaged TAFs on neighboring cells and alteration of stromal structure after cisplatin treatment. Findings suggested that while off-targeted NP damaged TAFs and inhibited tumor growth after an initial dose, chronic exposure to cisplatin NP led to elevated secretion of Wnt16 in a paracrine manner in TAFs. Wnt16 upregulation was then attributed to heightened tumor cell resistance and stroma reconstruction. Results attest to the efficacy of Wnt16 knockdown in damaged TAFs as a promising combinatory strategy to improve efficacy of cisplatin NP in a stroma-rich bladder cancer model.

1. Introduction

Cisplatin, a front line DNA alkylating agent, is a chemotherapeutic regime used to treat basal type muscle invasive bladder cancer [1]. Clinical application of cisplatin is limited by adverse effects including neuro- and nephrotoxicity [2–4]. Herein, nanoparticles (NPs) have been designed to improve the pharmacokinetics, facilitate the intratumoral accumulation and subsequently reduce adverse effects of cisplatin-based treatment [5–7]. In previous work we developed a novel cisplatin nanoformulation consisting of hydrophobic solid cisplatin cores surrounded by PEGylated cationic lipid corona for the delivery of cisplatin (cisplatin LPC NP, shorted as cisplatin NP) [8,9]. Anisamide, a ligand for the sigma receptor overexpressed on the surface of cancerous epithelial cells, was coated on the NP to enhance receptor mediated endocytosis [2,8,10]. Previous results proved that this novel cisplatin NP exhibited enhanced anticancer activity for the treatment of aggressive bladder cancer at low doses (cisplatin 1 mg/kg) compared to free cisplatin, which was completely ineffective at the same dose level [11].

Despite encouraging antitumor efficacy after initial treatment, drug resistant eventually contributes to ultimate treatment failure [11,12]. Efforts have been focused on combining cisplatin NP with another nanoformulated therapeutic regimen to inhibit DNA repair enzymes, cisplatin export and subsequently reverse tumor cell autonomous resistance [9,13,14]. However, inconsistencies between the *ex vivo* prediction of combinatory NP sensitivity and the *in vivo* therapeutic outcome suggest that stroma cells in the tumor microenvironment (TME) also play a key role in the *innate* resistance [15–18]. Stromal cells in the TME, including tumor associated fibroblasts (TAFs), macrophage and endothelial cells build a physical barrier within tumors by crosslinking the extracellular matrix (ECM) to inhibit penetration of the therapeutic NP [19,20]. They also mediate tumor cell-resistance by secreting growth inducing cytokines and growth factors [17,21–23]. Yet, the *innate* resistance from TME still fails to explain progressions from high treatment sensitivity in early stages to late stage therapeutic failure [18]. Findings suggest that acquired resistance in stromal cells may contribute to the long-term treatment failure [18]. DNA damage induced secretion of extracellular molecules was likely to explain the acquired resistance

Abbreviations: CM, conditioned medium; Cisplatin NP, without further indication, means anisamide targeted cisplatin NP; DRP, damage response program; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; FAPα, fibroblast activation protein alpha; GFP, green fluorescence protein; HUVECs, human umbilical vein endothelial cells; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; NPs, nanoparticles; RFP, red fluorescence protein; SRBC, stroma-rich bladder cancer model; siWnt NP, siRNA against Wnt16 encapsulated in a targeted LPH NP; siCont NP, control siRNA encapsulated in a targeted LPH NP; α-SMA, α-smooth muscle actin; TAFs, tumor associated fibroblasts; TME, tumor microenvironment; Wnt16, Wingless-type MMTV integration site family, member 16; PDX, patient derived xenograft.

* Corresponding author.
E-mail address: leafh@unc.edu (L. Huang).

http://dx.doi.org/10.1016/j.jconrel.2015.08.027
0168-3659/

[24,25]. In agreement with this theory, recent research indicates that damaged stromal cells secrete damage response program (DRP) molecules to promote the survival and growth of neighboring cells [18], and thus paracrinely influence treatment outcome [26,27].

Stromal cell populations damaged by cisplatin uptake control the secretion of DRP molecules. Therefore, intratumoral disposition, in particular the off-target distribution of NP in stroma cells was hypothesized to regulate drug resistance through secretion of DRP molecules. To reverse the cisplatin NP induced drug resistance, the blockade of DRP production and secretion was proposed in combination with cisplatin NP as a proof of concept strategy.

DRP molecules mainly consist of proteases and mitogenic growth factors [18,26,28]. Wingless-type MMTV integration site (Wnt) family molecules are considered as one of the major mitogenic growth factors that constituent DRP molecules [29]. Though little information has linked Wnt signaling to cisplatin induced resistance, abnormal Wnt signal activation promotes tumorigenesis, stemness and resistance in various tumors [30,31]. In addition, previous studies correlated Wnt16 expression and chemotherapy-induced DNA damage in prostate and breast cancer fibroblasts [18,32]. So, Wnt16 plays a potentially significant role in regulating the crosstalk between neighboring cells during DNA damage. Herein, Wnt16 is investigated as a potential DRP molecule for cisplatin mediated resistance and knockdown of Wnt16 was therefore proposed to overcome cisplatin induced resistance. Several studies have demonstrated functional blocking of Wnt-canonical β-catenin pathway using monoclonal antibodies or small molecule inhibitors [33]. However, undesired off-target effects have led to safety concerns in this approach [34,35]. RNA interference provides an alternative way to maintain the aforementioned specificity while also improving safety. Liposome-protamine-hyaluronic acid NP (LPH-NP) was used to encapsulate siRNA and was shown to be an effective delivery tool in various tumor models [36]. Therefore, based on previous claims, a combination therapy of cisplatin NP and LPH-NP delivered siRNA against Wnt16 (siWnt NP) was proposed to be the optimal treatment for of aggressive bladder cancer.

In the current study, a stroma rich bladder cancer model (SRBC) was established by co-inoculating human basal type bladder tumor UMUC3 with mouse NIH3 T3 fibroblasts. This model resembles bladder tumor patient samples in the components and in the morphology of the TME [11]. In the SRBC model, we investigated the off-target effects of cisplatin NP on TAF damage, the Wnt16 secretion level and antitumor efficacy. We also studied the role of cytokines, such as Wnt16 in the regulation of crosstalk between tumor cells and the TME. We conclude that targeting tumor-stroma regulatory cytokines in the TME along with NP-delivered chemotherapy could potentially overcome intratumoral off-target effects and improve treatment responses.

2. Material and methods

2.1. Materials

1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-(methoxy (polyethyleneglycol)-2000) ammonium salt (DSPE-PEG2000), 1,2-dioleoyl-3-trimethylammonium-propane chloride salt (DOTAP), and dioleoyl phosphatidic acid (DOPA) were obtained from Avanti Polar Lipids (Alabaster, AL). Cholesterol, hyaluronic acid (HA), protamine sulfate (fraction × from salmon), hexanol, triton-100, and cyclohexane were obtained from Sigma-Aldrich (St. Louis, MO). Cisplatin was purchased from Acros Organics (Fair Lawn, NJ). All the other chemicals were purchased from Sigma-Aldrich unless otherwise mentioned. DSPE-PEG-AA was synthesized based on the previous established protocols [10]. The mouse Wnt16 siRNA with sequence 5′-CCAACUACUG CGUGGAGAA-3′, the human Wnt16 siRNA with sequence of 5′-CCAA CUACUGUGUAGAAGA-3′ and the control siRNA with sequence 5′ AATCTCCGAACGTGTCACGT-3′ was purchased from Sigma-Aldrich.

2.2. Cell lines and animals

The human bladder transitional cell line UMUC3 was provided by Dr. William Kim (University of North Carolina at Chapel Hill, NC). The mouse embryonic fibroblast cell line NIH3T3 and the human umbilical vein endothelial cells (HUVECs) were purchased from UNC Tissue Culture Facility. UMUC3 and NIH3T3 were maintained in Dulbecco's Modified Eagle's Media (Invitrogen, Carlsbad, CA), supplemented with 10% fetal bovine serum (Sigma, St. Louis MO) or 10% bovine calf serum (Hyclone, Logan, Utah), penicillin (100 U/mL) (Invitrogen) and streptomycin (100 μg/mL) (Invitrogen). HUVECs were cultured in HuMEC basal medium (Invitrogen, Carlsbad, CA), supplemented with 10% fetal bovine serum (sigma, St. Louis, MO), bovine pituitary extract (Invitrogen, Carlsbad, CA) and HuMEC Supplement (Invitrogen, Carlsbad, CA). Female athymic Balb/C nude mice of 6–8 weeks old were provided by the University of North Carolina animal facility. All animal protocols were approved by the University of North Carolina at Chapel Hill's Institutional Animal Care and Use Committee.

2.3. Antibodies

Primary antibodies used for western-blot analysis and immunostaining included rabbit anti-fibronectin, anti-alpha-smooth muscle actin (αSMA), anti-fibroblast activation protein alpha (FAPα), GAPDH, anti-E cadherin and anti-N cadherin polyclonal antibodies (Abcam, Cambridge, MA), rabbit anti-beta catenin monoclonal antibody (Abcam, Cambridge, MA), rat anti-CD31 polyclonal antibody (Abcam, Cambridge, MA) and mouse monoclonal poly(ADP-ribose) antibody (PARP, Santa Cruz biotechnology, Inc.), rabbit polyclonal anti-Wnt16 antibodies (Santa Cruz biotechnology, Inc.). Secondary antibodies used for western-blot analysis and immunohistochemistry staining (IHC) included bovine anti-rabbit IgG-HRP, rabbit anti-mouse IgG-HRP (Sigma). And secondary antibodies used for immunofluorescence staining consists of FITC, Alexa Fluor® 555 and Alexa Fluor® 647 conjugated anti-rabbit and rat antibodies (Abcam, Cambridge, MA).

2.4. Preparation of cisplatin NP and LPH NP

Cisplatin NP and LPH NP were prepared and characterized as described previously [8,36]. Additional details of NP manipulation are provided in the Supplementary methods.

2.5. Cell treatments with cisplatin

NIH3T3 cells were pre-activated with 10 ng/mL TGFβ and treated with10 μm free cisplatin or cisplatin NP for 3 h before replacing into fresh full medium, and then left overnight (in total 2 days). After treatment, the conditioned medium (CM) was collected (Supplementary methods) and cells were rinsed 3× with PBS. Both CM and cells were used for western-blot assay (Supplementary methods) and ELISA assay (Supplementary methods) to detect the expression levels of Wnt16.

2.6. In vitro transfection of siWnt NP

UMUC3 cells or activated NIH3T3 cells were grown until 80% confluent in six-well plates. Then LPH NP loaded with siRNA against mouse Wnt16 (siWnt NP), human Wnt16 or control siRNA (siCont NP) was added to each well in the presence of OptiMEM medium with final concentration of 250 nmol/L. Medium was refreshed 4 h post-transfection. The remaining cells were treated for another 3 h with 10 μm cisplatin to boost the expression of Wnt16, then washed and left overnight. The knockdown efficiency and specificity of Wnt16 by siRNA were determined by western-blot analysis with GAPDH as a loading control.