Biochimie 94 (2012) 2391–2397

Contents lists available at SciVerse ScienceDirect

Biochimie

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

Research paper

Anti-KDEL-coated nanoparticles: A promising tumor targeting approach for ovarian cancer?

Florence Delie [a,1], Pascale Ribaux [b,1], Patrick Petignat [b], Marie Cohen [b,*]

[a] School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Quai Ernest Ansermet 30, 1211 Geneva 4, Switzerland
[b] Department of Obstetrics and Gynecology, Maternity, Geneva University Hospital, 1211 Geneva, Switzerland

ARTICLE INFO

Article history:
Received 27 April 2012
Accepted 8 June 2012
Available online 17 June 2012

Keywords:
Nanoparticle
Targeting
Ovarian cancer
GRP78
GRP94

ABSTRACT

The purpose of this study was to target ovarian cancer cells by coupling paclitaxel (Tx)-loaded nanoparticles (NPs-Tx) to antibodies against KDEL sequence, able to recognize GRP94 and GRP78 that are located at cell surface in cancer cells whereas they are in the endoplasmic reticulum in healthy cells. Tx-loaded poly (DL-lactic acid) nanoparticles coated with anti-KDEL antibodies (NPs-Tx-KDEL) were successfully prepared and characterized. Interaction between tumor cells and NPs-Tx or NPs-Tx-KDEL was observed by microscopy with fluorescently labeled NPs and the efficacy of the different formulations was compared by a viability assay.

Particles functionalized with monoclonal antibodies (mAb) showed a higher binding to the cells even though the internalization rate appeared limited. The effect of NPs-Tx-KDEL on cell viability (proliferation) was compared to Tx, NPs, NPs-Tx, anti-KDEL mAb or anti-KDEL mAb in combination with NPs-Tx in Bg-1 ovarian cell line. Our data indicate that NPs-Tx-KDEL significantly increase sensitivity of Bg-1 cells to Tx compared to other treatments.

This study confirms the interest of anti-cancer therapy by targeting cell surface GRP78 and GRP94 on cancer cells, and demonstrates the efficiency of coupling KDEL antibodies to NPs.

1. Introduction

Ovarian cancer is a curable disease when detected at an early stage; however, most patients are diagnosed at an advanced step when metastases have invaded the peritoneum. The survival rate is less than 20% despite aggressive surgery and chemotherapy [1]. Standard therapy is cytoreductive surgery, followed by combined chemotherapy [2]. About 75% of patients are chemo-sensitive and will present a complete response after initial treatment; however, the majority will relapse after a median time of 10–18 months [3,4]. To improve outcome for women with advanced ovarian cancer, it is essential to have new tools to increase both intensity and duration of the therapeutic effect.

Glucose-regulated proteins (GRP), GRP78 and GRP94, are chaperone proteins mainly located in the endoplasmic reticulum (ER) due to their carboxy-terminal ER retention domain defined by the KDEL amino acids sequence [5,6]. These proteins are involved in facilitating folding, maturation and transport of nascent peptides. They are normally present at low levels in adult cells but their expression is triggered by ER stress including glucose deprivation and hypoxia [7]. In numerous tumor cells, these proteins are overexpressed and associated to cancer growth and drug resistance [8–14]. Moreover, a fraction of these proteins is also found at cell surface of cancer cells where it can transduce extracellular stimuli to intracellular signals to promote cancer development [15,16]. For example, ligation of cell surface GRP78 with antibodies directed against the COOH-terminal domain of GRP78 was shown to suppress Ras/MAPK and PI3-kinase/AKT signaling while promoting caspase activation in human prostate cancer cells [17]. GRP78 autoantibodies purified from ovarian cancer patients' sera were also able to increase drug response and decrease invasiveness of ovarian cancer cells [18]. GRP94 also was suggested as a membrane receptor but its presence and role at cell surface have not been investigated in cancer biology.

Based on their role in cancer growth and drug resistance, GRP78 and GRP94 represent targets for solid tumor cancer therapy and

Abbreviations: DiO, dioctadecyloxacarbo-cyanine perchlorate; EDAC, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimidide; ER, endoplasmic reticulum; GRP, glucose-regulated protein; HER, herceptin; mAb, monoclonal antibodies; NP, nanoparticle; PLA, poly (DL-lactic acid); PVAL, poly (vinyl alcohol); sulfo-MBS, m-maleimidobenzoyl-N-hydroxy-sulfosuccinimide ester; TCEP, tris (2-carboxyethyl)-phosphine hydrochloride; Tx, paclitaxel.

* Corresponding author. Laboratoire d'Hormonologie, HUG Maternité, 30 bd de la Cluse, 1211 Genève 14, Switzerland. Tel.: +41 22 38 24 381; fax: +41 22 38 24 310.
E-mail address: marie.cohen@hcuge.ch (M. Cohen).

[1] These authors contributed equally to this work.

0300-9084/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.biochi.2012.06.010

a number of drugs inhibiting these proteins have already been developed [7]. Moreover, the presence of GRP78 at the surface of cancer cells but not of normal cells renders it very attractive to target cancer cells [19].

Indeed, the use of potent therapeutic antibodies against C-terminus part of GRP78 on drug loaded nanocarriers could both target cancer cells and inhibit tumor development. Paclitaxel (Tx) is one of the most efficient agents commonly used for the treatment of ovarian cancer. Tx stabilizes microtubules and thus interferes with their breakdown during cell division. However, it suffers from a poor solubility in water and a low therapeutic index associated with serious side effects. FDA approved formulations comprise Taxol® using Cremophor® EL and ethanol as solubilising agents or Abraxane®, a combination of Tx and albumin formulated as a suspension. Cremophor® EL presents severe side effects by itself, such as hypersensitivity reactions; Abraxane® is only approved for breast cancer. Various approaches have been proposed for the development of alternative formulations of Tx such as cosolvents, emulsions, cyclodextrins, pastes, implants and nanocarriers such as micelles, liposomes, microspheres or nanoparticles [20,21]. Polymeric nanoparticles (NPs) are nanocarriers with a size of 10–800 nm in which drugs may be entrapped in a polymeric matrix; therefore, a poorly-water soluble drug may be delivered as a suspension. Solid NPs are more stable in biological environment than liposomes and depending on the nature of the polymer, the release kinetic of the drug may be controlled. Poly (DL-lactic acid) (PLA) is a biodegradable and biocompatible polymer frequently chosen to manufacture NPs as it is already used for biomedical applications such as sutures or stents. Furthermore, due to enhanced permeability and retention effect (EPR), NPs formulation may promote a preferential distribution to tumor site. To further enhance selective drug distribution to target cells overexpressing specific antigens or receptors, the surface of NPs can be coated with specific ligands such as monoclonal antibodies (mAb). Therefore, drug carrier capacity and high loading efficiency of NPs can be combined with the ability of mAb to target tumor tissues. Recently, Tx-loaded NPs surface was functionalized with the mAb Herceptin® (HER) to obtain a targeted delivery system (NP-Tx-HER) for ovarian cancer cells overexpressing HER2 specific antigens. This drug delivery system has proven to be efficient *in vitro* and *in vivo* [22–24]. In order to investigate the relevance of GRP78 and GRP94 as targets to reach ovarian cancer cells, we have applied the same technology to prepare Tx-loaded immunoparticles coated with antibodies against the KDEL sequence present in C-terminus of both GRP78 and GRP94 to target invasive cells and increase response to apoptosis.

2. Methods

2.1. Materials

2.1.1. For NPs preparation

Poly (DL-lactic acid) (PLA, 100DL 4A, M_W 57 kD) was provided by Lakeshore Biomaterials, Inc (Birmingham, AL, USA). 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDAC), D(+)-Trehalose dihydrate, phosphate buffer saline (PBS), poly-L-Lysine solution, 0.1% (w/v) were from Sigma (Buchs, Switzerland). m-Maleimidobenzoyl-N-hydroxy-sulfosuccinimide ester (sulfo-MBS), tris (2-carboxyethyl)-phosphine hydrochloride (TCEP) and D-salt dextran plastic columns were supplied by Pierce (Rockford, IL, USA). Dioctadecyloxacarbo-cyanine perchlorate (DiO) was from Molecular Probes (Leiden, The Netherlands). Poly(vinyl alcohol) (PVAL, Mowiol 4–88) was purchased from Hoechst (Frankfurt/M, Germany). Paclitaxel (Tx) was obtained from Cfm Oskar Tropitzsch (Marktredwitz, Germany).

2.1.2. For membrane protein extraction

The complete protease inhibitor cocktail tablets were purchased from Roche Diagnostics GmbH (Mannheim, Germany). The assay to measure protein concentration was provided by BIO-RAD (Munich, Germany).

2.1.3. For Western blotting

Pre-stained molecular weight markers (Novex® Sharp) were from Invitrogen (Carlsbad, CA, USA). Rabbit anti-GRP78 antibodies (GL-19) were from Sigma (Saint-Louis, MO, USA), mouse anti-KDEL antibodies from Assay Designs (Ann Arbor, MI, USA) and mouse anti-GAPDH antibodies from Chemicon (Billerica, MA, USA). Secondary antibodies were goat anti-mouse IgG-HRP conjugated from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and goat anti-rabbit IgG-HRP conjugated from BIO-RAD (Hercules, CA, USA). The enhanced chemiluminescence (ECL) detection kit was purchased from GE Healthcare (Buckinghamshire, UK).

2.1.4. For immunofluorescence

Rhodamine-phalloïdin was from Invitrogen and Vectashield® from Vector Laboratories, Inc. (Burlingame, CA, USA).

2.1.5. For cell viability assay

Cell proliferation reagent (WST-1) was provided by Roche Diagnostics GmbH (Indianapolis, IN, USA).

2.2. Tx-loaded NPs preparation and anti-KDEL grafting

Tx-loaded NPs (NPs-Tx) were prepared by a salting-out process as described previously [22,25]. Briefly, Tx dissolved in acetone was added to a solution of PLA in acetone (drug to polymer ratio: 1:10). To determine the cellular localization of NPs, DiO 0.01% (w/w) was added in the organic phase as fluorescent probe. The organic phase was mixed under vigorous stirring at 45 °C with an aqueous phase containing 15% (w/w) PVAL used as surfactant and 60% (w/w) magnesium chloride hexahydrate. The miscibility of the two phases was prevented by the high concentration of salt. Then, 20 ml of pure water was added to the NPs suspension and stirring was maintained for 10 min. NPs were recovered by centrifugation (Optima® XL-100K, rotor 70.1 Ti Centrifuge, Beckman Coulter Inc., Fullerton, CA, USA), washed twice with pure water to remove unencapsulated drug, PVAL and magnesium salt. After preparation and purification, NPs were lyophilized (Edwards, Modulyo, Oberwil, Switzerland) for 2 days in presence of trehalose (30% w/w was chosen after optimization) as a lyoprotector and stored at 4 °C until use.

Grafting of the anti-KDEL antibodies was obtained using a carbodiimide method as already described [22]. In a first step, the free carboxyl groups of the polymer at the NPs surface were thiolated, then the covalent attachment of antibodies to thiolated NPs is obtained *via* a sulfo-MBS cross-linker. Briefly, PLA NPs were suspended in water and reaction initiated by adding, consecutively a solution of EDAC then cystein solution. The final suspension was stirred under mild conditions for 24 h at room temperature. Remaining EDAC and non-reacted cystein was removed by centrifugation. The NPs were then resuspended in purified water before reduction of disulfide bonds by a solution of TCEP. Finally, the NPs were purified by centrifugation and freeze-dried in presence of trehalose to avoid aggregation. In the following step, the anti-KDEL antibodies were activated with sulfo-MBS in PBS (pH 7.4). After removing non-reacted sulfo-MBS by size exclusion chromatography using desalting columns, the activated ligand was incubated with NPs and gently shaken at room temperature. Unconjugated ligand was removed by centrifugation.