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Increasing the affinity of cationized polyacrylamide-paclitaxel nanoparticles towards colon cancer cells by a surface recognition peptide

Sanjay Tiwari[1], Boaz Tirosh, Abraham Rubinstein*

The Hebrew University of Jerusalem, Faculty of Medicine, School of Pharmacy Institute for Drug Research, P.O. Box 12065, Jerusalem, 91120, Israel

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Abbreviations:
CPAA
cationized polyacrylamide
FACS
fluorescence-activated cell sorting
FITC
fluorescein isothiocyanate
CPAA-Pep
CPAA conjugated to VRPMPLQ
CPAA-ScrPep
CPAA conjugated to the scrambled sequence VPQLRPM
DSC
differential scanning calorimetry
NP
nanoparticles
PLX
paclitaxel

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Cationized polyacrylamide nanoparticles
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VRPMPLQ recognition peptide
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ABSTRACT

Nanoparticles of cationized polyacrylamide (CPAA NP), decorated with the recognition peptide VRPMPLQ (to produce CPAA-Pep NP), were prepared, characterized and tested biologically. They were designed to target dysplasia regions in the colon, characterized by overexpressed sialic acid. This targetability was augmented by the addition of VRPMPLQ. Their mean hydrodynamic size was 137 nm with narrow size distribution and positive zeta potential. When incubated with three types of colon cancer cells, a 10-fold increase in the cell's uptake was found for the CPAA-Pep NP compared with the CPAA NP. The use of a scrambled sequence of the VRPMPLQ peptide and competition studies, employing excess of the free peptide verified the specific nature of the NP cellular uptake. Nanoparticles loaded with paclitaxel with and without VRPMPLQ indicated an improved pro-apoptotic activity of the CPAA-Pep NP. It is speculated that both positive charge and the presence of VRPMPLQ could serve as an improved strategy to deliver nanoparticles loaded with cytotoxic drugs for the treatment of colon cancer.

1. Introduction

Identification of reliable biomarkers on cancer cells is a major challenge in the design of targeted cancer therapy. Such viable markers enable a focused therapy at the tumor site, sparing adverse effects, improving the efficacy and tolerability of cytotoxic drugs. In addition, they can be used for diagnostics and theranostics. The prototype example is the erbB2 receptor that undergoes upregulation in certain types of cancers (e.g. breast cancer), in which targeting is achieved by specific antibodies directed against the ectodomain of the protein (Nagy et al., 1999). However, the use of antibodies for homing is restricted in the design of nanocarriers aimed at solid tumors due to their large molecular dimensions, immunogenicity and complexity in large scale production settings. Thus, small peptides are attractive opportunities.

Several peptides have been identified as potential targeting moieties to solid tumors. Shadidi and coworkers reported that the peptide sequence LTVSPWY, selected from a peptide library, is capable to specifically bind to breast cancer cells (Shadidi and Sioud, 2003). Other recognition moieties were utilized to direct nano vehicles, such as liposomes, to colon cancer (Garg et al., 2009). The peptide VRPMPLQ, isolated from a phage display library, was screened for specific interaction with freshly isolated adenocarcinoma of the colon. Interestingly, this peptide, when conjugated to a fluorophore, allowed the visualization of colon malignancies by confocal microendoscopy (Hsiung et al., 2008). We have further explored the ability of this peptide to direct water-soluble polymers to colon carcinoma for diagnostic purposes

* Corresponding author.
E-mail address: avrir@ekmd.huji.ac.il (A. Rubinstein).
[1] Current Address: Uka Tarsadia University, Maliba Pharmacy College, Gopal-Vidyanagar Campus, Surat, 394350, Gujarat, India.

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(Bloch et al., 2016, 2015). However, the possibility that this peptide can also be used to target colon cancer with drug-loaded nanoparticles (NP) has not been studied.

The mitotic inhibitor paclitaxel (PLX), a prototype of the taxane family, interferes with cell division via interfering with microtubule-driven cell depolarization and it is widely used in a broad range of cancers including lung, ovarian, breast and pancreatic cancers (Schiff et al., 1979). Its poor water-solubility, however, is an obstacle. It is commonly injected in a mixture of Cremophor and ethanol. Nevertheless, due to severe adverse effects of the Cremophor, a slow infusion of the drug is commonly applied. An intriguing solution to PLX mode of administration would be its incorporation into nanocarriers due to their concomitant ability to improve solubility of hydrophobic drugs with a relatively low toxicity (Ma and Mumper, 2013). Indeed, albumin-bound NP of PLX (Abraxane®) have already been approved for the treatment of metastatic breast cancer and non-small cell lung cancer. Once loaded into NP the pharmacodynamics of PLX could even be improved by decorating the NP with recognition moieties that lead to enhanced uptake by pre-defined target cancer cells and reduce overall toxicity of the drug. Thus, the cytotoxicity of PLX NP was increased by the addition of transferrin (Sahoo et al., 2004), RGD (Wang et al., 2011) and folic acid (Ma and Mumper, 2013; Zhao et al., 2010). Recently, PLX nanocrystals, entrapped in crosslinked hyaluronic acid gel were suggested for the spatial treatment of peritoneal carcinoma from ovarian origin (Sun et al., 2016).

In this study we have used PLX NP as a model to explore the uptake into colon cancer cells. We report on the design of novel colon cancer targetable NP made of cationized polyacrylamide (CPAA), decorated with the recognition peptide, VRPMPLQ and loaded with PLX. The positive charge of the polymer was found to be essential for its targetability owing to the over-expressed sialic acid in colon cancer cells (Azab et al., 2008; Bloch et al., 2012). The specific goals of the study were to, (***a***) prepare and characterize the NP; (***b***) examine their binding to different colon cancer cells; (***c***) test whether the bound NP internalize into the cells and elucidate the mode of internalization; and (***d***) test if entrapping PLX into NP decorated with the recognition peptide (CPAA-Pep NP) improved its apoptotic efficacy. Our data indicate a clear advantage of the CPAA NP when decorated with the recognition peptide in interacting with, penetrating into and eventually conferring cytotoxicity to colon cancer cells.

2. Materials and methods

2.1. Materials and equipment

All materials were purchased from Sigma, St. Louis, MO, USA, unless otherwise stated. All solvents (Bio Lab, Jerusalem, Israel) were of analytical grade; Water was purified by reverse osmosis; Sodium carbonate anhydrous, *N*,*N*-diisopropylethylamine (DIEA), piperidine, trifluoroacetic acid (TFA) and *N*,*N*-dimethylformamide (DMF) were purchased from Bio Lab, Jerusalem, Israel; Acryl amide and triethylsilane were purchased from Fluka, Seelze, Switzerland; *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate (HATU) was purchased from Anaspec, Fremont, CA, USA; Benzoyl peroxide and molecular sieve (0.4 nm beads) were purchased from Merck, Darmstadt, Germany; 2,6-Dimethylpyridine (Lutidine) was purchased from ABCR GmbH, KG, Karlsruhe, Germany. Linear polyethylene imine (Mw = 2.5 KDa and 250 KDa) was purchased from Polysciences Warrington, PA, USA. All amino acids were purchased from Gl Biochem, Shanghai, China. DAPI Fluoromount-G was purchased from Southern Biotech, Al, USA; Deuterium oxide (D_2O), dimethyl sulfoxide (DMSO) D_6chloroform D andmethanolD_4 were purchased from Cambridge Isotope Laboratories, USA.4-(dimethylamino) pyridine was purchased from Alfa Aesar, Ward Hill, MA USA; Sorbitan monooleate (Span 80) and ethylenediamine tetraacetic acid (EDTA) disodium salt dehydrate were purchased from J.T. Baker PA, USA; Potassium chloride analytical and sodium chloride analytical were purchased from Frutarom, Haifa, Israel; Sodium carbonate anhydrous, granular was purchased from Mallinckrodt Chemicals, Phillipsburg, NJ; 1-Ethyl-(3-dimethylaminopropyl) carbodiimide hydrochloride, anhydrous (EDAC) was purchased from Chem-Impex International Wood Dale, IL, USA. Pentynoic acid, neuraminidase and Annexin V-FITC apoptosis detection kit were purchased from Sigma Aldrich, USA. Mito Tracker kit was purchased from Thermo Fisher Scientific, Waltham, MA, USA.

Proton NMR was recorded on a 300 MHz Brucker NMR using deuterated solvents as internal standards. Mass analysis of the CPAA and the CPAA-Pep polymer was performed by LCMS (Finnigan LCQ duo, ThermoQuest, CA, USA).The gel permeation chromatograph (Malvern Instruments, UK) was equipped with multi-angle laser light scattering detector (DAWN-F DSP Spectrophotometer, Wyatt, USA). The gas chromatograph (GC) was Agilent 7890B/7200 GC/Q-TOF), equipped with an auto sampler (CTC Analytics, Zwingen, Switzerland) and Mass Hunter B.07.00 data acquisition software. The Lyophilizer was Epsilon 2–6 DLSC plus (Martin Christ, GmbH, Germany). Differential scanning calorimetry (DSC) analysis was performed in Mettler Toledo DSC1, Greifensee, Switzerland. Dynamic light scattering was conducted by Zetasizer (Nano ZS, Malvern Instruments, UK). The transmission electron microscope (TEM) was JEM-1400 Plus (JEOL, Tokyo, Japan). Images were analyzed with the aid of the ImageJ software (NIH, USA). The atomic force microscope (AFM) was Dimension 3100 SPM, Bruker, USA. X-ray powder diffraction measurements were performed in the D8 ADVANCE diffractometer (Bruker AXS, Karlsruhe, Germany) with a secondary graphite monochromator and 2°Soller slits. The HPLC consisted of HP 1050 pump, equipped with a UV–vis detector and Chemstation data acquisition software. Cell sorting analysis was conducted on CytoFLEX flow cytometer (Beckman Coulter, UK). Confocal microscopy was conducted on confocal laser scanning microscope (Zeiss 710, Omaha, USA). The images were analyzed by the ZEN 2.1 software (Zeiss, Oberkochen, Germany). The dual staining images for colocalization were analyzed by Image-Pro Ver 7 (Media Cybernetics, Rockville, MD, USA).

2.2. Cell lines and culturing

HT-29, SW-480 (both represent Stage B colon cancer severity in the Duke staging system), SW-620 (represents Stage D colon cancer severity in the Duke staging system, with increased expression of sialic acid) were purchased from American Type Culture Collection, Manassas, Virginia, USA. All cells were cultured (37 °C, 5% CO_2), in DMEM medium supplemented with 10% fetal calf serum, 2 mM L-glutamine and 1% Pen/Strep solution (Biological Industries, Beit Haemek, Israel).

2.3. Cationized polyacrylamide preparation

The synthesis of *N*-acryloyl, *N*-(*tert*-butyl-carbonyl)-diaminoethane (the cationic monomer) and its polymerization to cationized polyacrylamide (CPAA) by radical polymerization was conducted according to the previously published procedure (Bloch et al., 2016). The molecular weight of the CPAA was determined by gel permeation chromatography. It ranged between 33 and 56 KDa (M_w/M_n 2.39).

2.4. Attachment of the recognition peptide

The recognition sequence valine-arginine-proline-methionine-proline-leucine-glutamine (VRPMPLQ) and its scrambled sequence, VPQLRPM were prepared, using solid phase peptide synthesis as described before (Bloch et al., 2015). VRPMPLQ or VPQLRPM were conjugated (15 mol%) to the CPAA backbone by a click reaction, using pentynoic acid as a linker, to give CPAA-Pep or CPAA-ScrPep (Bloch et al., 2016, 2015). The CPAA-Pep was characterized by ^{1}H NMR, in which typical peaks appeared in the aliphatic at 7.67 ppm (attributed to the hydrogen of the triazole ring). HSQC NMR spectrum showed a peak