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Research paper

Cancer cell death induced by nanomagnetolectin

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ABSTRACT

Magnetic nanoparticles represent a new paradigm for molecular targeting therapy in cancer. However, the transformative targeting potential of magnetic nanoparticles has been stymied by a key obstacle-safe delivery to specified target cells in vivo. As cancer cells grow under nutrient deprivation and hypoxic conditions and decorate cell surface with excessive sialoglycans, sialic acid binding lectins might be suitable for targeting cancer cells in vivo. Here we explore the potential of magnetic nanoparticles functionalized with wheat germ lectin (WGA) conjugate, so-called nanomagnetolectin, as apoptotic targetable agents for prostate cancer. In the presence of magnetic field (magnetofection) for 15 min, 2.46 nM nanomagnetolectin significantly promoted apoptosis (~12-fold, p value <0.01) of prostate cancer cells (LNCaP, PC-3, DU-145) compared to normal prostate epithelial cells (PrEC, PNT2, PZ-HPV-7), when supplemented with 10 mM sialic acid under nutrient deprived condition. Nanomagnetolectin targets cell-surface glycosylation, particularly sialic acid as nanomagnetolectin induced apoptosis of cancer cells largely diminished (only 2 to 2.5-fold) compared to normal cells. The efficacy of magnetofected nanomagnetolectin was demonstrated in orthotopically xenografted (DU-145) mice, where tumor was not only completely arrested, but also reduced significantly (p value <0.001). This was further corroborated in subcutaneous xenograft model, where nanomagnetolectin in the presence of magnetic field and photothermal heating at ~42 °C induced apoptosis of tumor by ~4-fold compared to tumor section heated at ~42 °C, but without magnetic field. Taken all together, the study demonstrates, for the first time, the utility of nanomagnetolectin as a potential cancer therapeutic.

1. Introduction

Altered cell-surface glycosylation has long been recognized as distinguishing feature of cancer in general and can be exploited for the new and improved biomarkers and therapeutic options (Pinho and Reis, 2015; Freeze, 2013; Kang et al., 2010; Fuster and Esko, 2005). Sialic acids occupy the terminal position of glycan chains of glycoproteins and glycolipids and contribute to the huge range of glycan structures that mediate cell surface biology. Altered expression of certain sialic acid types or their linkages is closely associated with cancer cellular adhesion, migration and metastasis. The ability to targeting aberrant sialylation in cancer tissues by non-invasive means in vivo would provide tremendous advantages for diagnostic and therapeutic purposes. Therefore, new approaches for molecular targeting, particularly glycan targeting, are increasingly being used to understand the complexity, diversity and in vivo behaviour of cancers.

Although a variety of approaches for delivering drugs to cancer cells such as delivery of plasmid DNA and siRNA through viral and non-viral vectors are available (Zhao et al., 2016; Wittrup and Lieberman, 2015; Jones et al., 2013), the use of nanotechnology in medicine is rapidly spreading and is being applied to improve diagnosis and therapy of diseases through effective delivery of drugs or imaging agents to target cells at disease sites (Smith et al., 2013; Davis et al., 2010; Park et al., 2010; Petros and DeSimone, 2010). Nanoparticle encapsulated chemotherapeutic drugs have

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been shown to reduce systemic toxic side effects from generalized systemic distribution. Magnetic drug targeting offers an opportunity to treat malignant tumors loco-regionally without systemic toxicity. The loco-regional cancer treatment with magnetic drug targeting needs several features to be considered: (i) the particles should be of a size that allows sufficient attraction by the magnetic field and their introduction into the tumor or into the vascular system surrounding the tumor; (ii) the magnetic fields should be of sufficient strength to be able to attract the magnetic nanoparticles into the desired area; (iii) the particles complex should deliver and release a sufficient amount of anticancer agents; and (iv) the method of treatment should have good access to the tumor vasculature (Alexiou et al., 2000, 2003, 2006). During past years, nanoparticle-based chemotherapeutics for various cancer and diseases have been approved for clinical use and many more are being studied in clinical trials (Torchilin, 2014; El-Sayed et al., 2006; Min et al., 2015; Barenholz, 2012). In recent years, the efficacy of these nanoparticle-based chemotherapeutics has been further improved by employing magnetic field such that magnetic field holds chemotherapeutic agent at the desired site of activity, thus increasing efficacy and diminishing systemic toxicity (Mura et al., 2013; Park, 2013; Stanley et al., 2012; Ruiz-Hernaández et al., 2011; Hoare et al., 2011).

Going back to the altered glycosylation in cancer cell surface, numerous studies indicated connections between glycosylation and abnormal glucose metabolism associated with most types of cancer (Ohtsubo and Marth, 2006). As cancer cells experience nutrient deprivation and hypoxia during oncogenesis, the resulting metabolic reprogramming, which endows cancer cells with the ability to obtain nutrients during scarcity, constitutes an "Achilles' heel" that can be exploited by metabolic glycoengineering (MGE) strategies to develop new diagnostic methods and therapeutic approaches (Krambeck et al., 2009; Djansugurova et al., 2012; Badr et al., 2013, 2014). We recently, in vitro, demonstarted that breast cancer cells can efficiently scavenge sialic acid under nutrient deprived condition and decorate cell surface sialoglycans with new epitopes, preferentially with α2,3 linked sialic acid, that can be targeted for diagnostic as well as therapeutic (so called theranostic) applications (Badr et al., 2015a,b, 2016). Therefore, the continued elucidation of lectin–glycan selectivity under specified metabolic conditions is critical step to target cancer-specific glycan alterations. Of a few sialic acid specific lectins that can target cancer sialoglycans, wheat germ agglutinin (WGA) may be suitable as this is obtained from a natural dietary source.

As a first step, we used WGA to target sialoglycans of cancer cells under nutrient deprived condition. We took advantage of the benefit of nanoparticles and magnetic field and prepared, for the first time, magnetic nanoparticles conjugated with the WGA, termed as "nanomagnetolectin". In this paper, we described the preparation of the nanomagnetolectin and its effect on cancer cell apoptosis in in vitro and in mice xenograft models. Our data suggest that nanomagnetolectin kills cancer cells and completely abolished tumor in mice indicating a potential use of the nanomagnetolectin for cancer therapeutics.

2. Materials and methods

2.1. Materials

Wheat germ agglutinin (WGA) was purchased from Vector Laboratories (Burlingame, CA, USA). Fluorescein isothiocyanate (FITC) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Magnetic nanoparticles (Nano-screen-fluidMAG/B-ARA), Magnetic separator (MagnetoPURE PM-20), Magnetic 96-well format (MagnetoFACTOR-96 plate) and CombiMAG-1000 particles were from Chemicell (Berlin, Germany). Sialic acid (N-acetyl-5-neuraminic-acid, Neu5Ac), was from Santa Cruz Biotechnology (San Diego, CA, USA). FITC BrdU Apoptosis Detection Kit was from BD Biosciences (San Jose, CA, USA). FITC-fluorescein conjugated anti-digoxigenin, MES (2-[N-Morpholino] ethanesulfonic acid) and EDC (1-ethyl-3-[3-dimethylaminoproply] carbodiimide) were from Sigma-Aldrich (Carlsbad, CA, USA). Phosphate-buffered saline (PBS, 137 mM sodium chloride, 2.7 mM potassium chloride, 4.3 mM disodium phosphate, 1.4 mM monopotassium phosphate, pH 7.5) was obtained from Technova (Hollister, CA, 95023 CA USA). Cell culture reagents were from Life Technologies Corporation (San Diego, CA, USA); all other chemicals were of analytical grade and purchased from Sigma-Aldrich.

2.2. Preparation of nanoparticles

2.2.1. Nanoparticles preparation and lectin conjugation

Aliquot of magnatic nanoparticles (50 nM based on Iron oxide Fe_3O_4) in a 2 ml standard Eppendorf tube was washed twice with 1 ml of coupling buffer MES [0.1 M 2-(N-Morpholino) ethanesulfonic acid, pH 5.0] using magnetic separator (MagnetoPURE PM-20). After discarded MES buffer, freshly prepared of coupling agent EDC (10 mg [1-ethyl-3-(-3-dimethylaminoproply) carbodiimide] in 0.2 ml MES, pH 5.0) was added to the washed particles and vortexed thoroughly for 30 s. WGA lectin (3–10 nM) or its FITC-fluorescein conjugates were coupled to the activated magnatic nanoparticles simultaneously and the resulting suspension was shaken gently for two hours at room temperature. At the end of the incubation time, the uncoupled WGA lectin was removed by washing the particles three times with 1 ml PSB and the WGA lectin conjugated magnetic nanoparticles (so called nanomagentolectin) kept at 4 °C until use. Concentration of lectin in the lectin-nanoparticle conjugate or nanomagnetolectin was determined by the method previously described (Paschkunova-Martic et al., 2005) after correcting the background with unlabeled magnetic nanoparticles. Berifly, three soultions were prepared: Amido Black 10 B dye (100 mg/ml, solution A); mixture of methanol and acetic acid (25:75 v/v, solution B, washing solution); and solution C was 1 M NaOH. The nanomagnetolectin samples were dissolved in 1 ml of deionized water, and mixed intensively with 1 ml of solution A. The mixture was then vortexed for 5 min and centrifuged at 5000×g for 5 min at 0 °C. The pellets were collected and washed several times with solution B before air dry at RT. The dried samples were dissolved in 3 ml of solution C, and determined by UV–vis spectroscopy at 625 nm. To prepare WGA-FITC, FITC solution (1 mg/ml in DMSO) was slowly added to WGA solution (2 mg/ml in 0.1 M sodium carbonate buffer, pH 9.0) (50 μl FITC solution per ml of WGA solution) in the dark at 4 °C and the resulting conjugate was separated on a desalting column based on the FITC manufacturer's instructions.

2.2.2. Nanoparticles size and size distribution

The free nanoparticles and the complexed nanomagnetolectins size and size distribution were determined by laser light scattering with Zeta Potential/Particle Sizer PSS/NICOMP 380 ZLS from Particle Sizing Systems, Inc. (Santa Barbara, CA, USA) at a fixed angle of 90° at 23 °C. In brief, the free nanoparticles and formulated nanomagnetolectin particles were suspended in filtered deionized water and sonicated to prevent particle aggregation and to form uniform dispersion of nanoparticles. The narrow size distribution was given by the polydispersity index. The lower the value is, the narrower the size distribution or the more uniform of the nanoparticles sample. The data represent the average of six measurements.