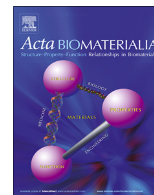
Acta Biomaterialia 58 (2017) 205–213

Contents lists available at ScienceDirect

Acta Biomaterialia

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



Full length article

Interleukin-13 conjugated quantum dots for identification of glioma initiating cells and their extracellular vesicles

A.B. Madhankumar [a,*], Oliver D. Mrowczynski [a], Suhag R. Patel [a], Cody L. Weston [a], Brad E. Zacharia [a], Michael J. Glantz [a], Christopher A. Siedlecki [b], Li-Chong Xu [b], James R. Connor [a]

[a] Department of Neurosurgery, Pennsylvania State University College of Medicine, Hershey, PA 17033, United States

[b] Department of Bioengineering, Pennsylvania State University College of Medicine, Hershey, PA 17033, United States

ARTICLE INFO

Article history:
Received 21 January 2017
Received in revised form 9 May 2017
Accepted 1 June 2017
Available online 3 June 2017

Keywords:
Interleukin-13
Quantum dots
Exosomes
Electron microscopy
Atomic force microscopy
Cerebrospinal fluid
Stem cells

ABSTRACT

Cadmium selenide (CdSe) based quantum dots modified with polyethylene glycol and chemically linked to interleukin-13 (IL13) were prepared with the aim of identifying the high affinity receptor (IL13Rα2) which is expressed in glioma stem cells and exosomes secreted by these cancer stem cells. IL13 conjugated quantum dots (IL13QD) were thoroughly characterized for their physicochemical properties including particle size and surface morphology. Furthermore, the specific binding of the IL13QD to glioma cells and to glioma stem cells (GSC) was verified using a competitive binding study. The exosomes were isolated from the GSC conditioned medium and the expression of IL13Rα2 in the GSC and exosomes was verified. The binding property of IL13QD to the tumor associated exosomes was initially confirmed by transmission electron microscopy. The force of attraction between the quantum dots and U251 glioma cells and the exosomes was investigated by atomic force microscopy, which indicated a higher force of binding interaction between the IL13QD and IL13Rα2 expressing glioma cells and exosomes secreted by glioma stem cells. Flow cytometry of the IL13QD and exosomes from the culture media and cerebrospinal fluid (CSF) of patients with glioma tumors indicated a distinctly populated complex pattern different from that of non-targeted quantum dots and bovine serum albumin (BSA) conjugated quantum dots confirming specific binding potential of the IL13QD to the tumor associated exosomes. The results of this study demonstrate that IL13QD can serve as an ex vivo marker for glioma stem cells and exosomes that can inform diagnosis and prognosis of patients harboring malignant disease.

Statement of Significance

Functionalized quantum dots are flexible semiconductor nanomaterials which have an immense application in biomedical research. In particular, when they are functionalized with biomolecules like proteins or antibodies, they have the specialized ability to detect the expression of receptors and antigens in cells and tissues. In this study we designed a cytokine (interleukin-13) functionalized quantum dot to detect a cancer associated receptor expressed in cancer stem cells and the extracellular vesicles (exosomes) secreted by the cancer cells themselves. The binding pattern of these cytokine modified quantum dots to the cancer stem cells and exosomes alters the physical properties of the complex in the fixed and suspended form. This altered binding pattern can be monitored by a variety of techniques, including transmission electron microscopy, atomic force microscopy and flow cytometry, and subsequent characterization of this quantum dot binding profile provides useful data that can be utilized as a fingerprint to detect cancer disease progression. This type of functionalized quantum dot fingerprint is especially useful for invasive cancers including brain and other metastatic cancers and may allow for earlier detection of disease progression or recurrence, thus saving the lives of patients suffering from this devastating disease.

© 2017 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

1. Introduction

The cerebrospinal fluid (CSF) and serum of brain tumor patients often exhibit an abnormal vesicle and associated membrane protein expression pattern [1]. Tumor cell secretome and extracel-

* Corresponding author.
E-mail addresses: abm10@psu.edu, abmkumar@gmail.com (A.B. Madhankumar).

http://dx.doi.org/10.1016/j.actbio.2017.06.002
1742-7061/© 2017 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

lular vesicle (exosomes) based biomarkers in the biofluid have recently been explored in neuro-oncology research, to evaluate prognosis and determine therapeutic response [2,3]. Exosomes play a significant role in cancer progression and invasion in central nervous system (CNS) malignancies by altering the tumor microenvironment [1,4,5]. The nature and composition of proteins in the exosomes may provide the earliest indication of disease relapse or progression in the CNS. The presence of exosomes in CSF samples may provide evidence for the presence of glioma tumor cells or even cancer initiating cells (also known as glioma stem cells) from glioblastoma in the brain.

In order to improve detection of glioma initiating cells and tumor associated exosomes, we expanded our nanotherapeutic approach by conjugating the interleukin 13 (IL13) protein to quantum dots. Quantum dots (QD) are cadmium and selenium based semiconductor nanoparticles ranging in size from 1 to 20 nm. These particles have a variety of applications in physiological systems from drug delivery to disease diagnosis [6–8]. Tumor cells secrete a variety of soluble factors and extracellular vesicles (exosomes) in the serum and in cerebrospinal fluid very early in the course of tumor recurrence, progression and as a prelude to metastasizing. For example, Interleukin 13 Receptor alpha 2 (IL13Rα2) is one type of oncogenic receptor expressed in several malignancies including GBM, ovarian cancer and even in recurrent glioma [9]. IL13Rα2 expression is a marker of tumor malignancy and invasiveness [10–12]. IL13 has specific binding affinity to IL13Rα2, which is overexpressed in cancer cells compared to its normal physiological receptor, IL13Rα1. In a previous study, we demonstrated the therapeutic potential of this marker in the form of IL13 linked nanovesicles [13,14]. In the present study, we investigate the interaction of IL13QD with glioma stem cells and exosomes to determine their suitability as a unique detection tool.

The goal of this study is to develop a method for identifying exosomes secreted by cancer cells including stem cells using ligand conjugated quantum dots. These findings, in future, would aid in the early detection of glioblastoma recurrence and to distinguish recurrence from pseudoprogression [15,16]. If successful, this could also provide the first practical screening test for populations at increased risk of developing brain tumors [15].

2. Materials and methods

2.1. Materials

Cadmium selenide (CdSe) quantum dots surface modified with polyethylene glycol (PEG) and carboxylic acid functional groups used for our studies were purchased from Ocean Nanotechnology, Inc. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), a water soluble carbodiimide was purchased from Thermoscientific, Inc. Sephadex G25M columns were purchased from GE Healthcare, Inc. YM-30 centrifugal concentrators were from Amicon, Inc. Fluorescent dye PKH67 was purchased from Sigma Aldrich, Inc. Human glioma cells U251 were obtained from American type culture collection (ATCC) and the human glioma stem cells T3691 and T387 were provided by Dr. Jeremy Rich, Cleveland Clinic Lerner Research Institute. Human IL13 protein (recombinant) was expressed in E. Coli and purified in our laboratory as described by us previously [1]. Cerebrospinal fluid (CSF) was collected from the brain tumor patients admitted to Penn State Hershey Medical Center after obtaining IRB approval.

2.2. Preparation and characterization of IL13 conjugated quantum dots

The quantum dot was conjugated to IL13 or BSA protein by EDC chemistry using EDC as conjugating agent [16,17]. Briefly, the carboxylated quantum dot at 2 μM concentration was reacted with 10 equivalents of IL13 protein in the presence of EDC for 1 h at room temperature under mild agitation. The excess unreacted EDC in the reaction mixture was removed by passage through a Sephadex G25M column. The eluted fractions from the column were concentrated in an YM-30 centriprep concentrator. The conjugation of the nanoparticles was confirmed by agarose gel electrophoresis and dot blots. The particle size before and after conjugation was also verified using a zetasizer (Malvern Nano ZS).

2.3. Surface morphology

The images indicative of the surface morphology of quantum dots were obtained using a Multimode Atomic Force Microscopy (AFM) with a Nanoscope IIIa control system (software version 5.12r3, Digital Instruments, Santa Barbara, CA) operating under tapping mode (intermittent contact). The image acquisition was performed under ambient conditions.

2.4. In vitro studies

2.4.1. Binding of IL13QD to glioma cells

In vitro binding experiments were performed using IL13QD and unconjugated QD on U251 human glioma cells and CD133 positive glioma initiating cells (designated T3691). To demonstrate the expression of IL13Rα2 receptor in T3691 GSC and in U251 glioma cells, the cells were cultured in monolayer using geltrex matrix, a basement membrane extract (Gibco, Inc.). T3691 cells were also cultured as aggregated neurospheres and subjected to immunocytochemistry by treating the cells with antibody for IL13Rα2 and subsequent treatment with Alexa Fluor 488 conjugated secondary antibody. For the binding studies 20,000 cells per well were plated in glass chamber slides and cultured at 37 °C. These cells were exposed to 100 fold molar excess of human recombinant IL13 protein, with respect to the QD concentration for 1 h. These cells were then exposed to QD at 20 nM concentration at 4 °C for 10 min (U251 cells) and 30 min (T3691 cells), respectively. Subsequently, the excess quantum dots with media from the cells were removed and replaced with fresh media; and, incubated at 37 °C for 1 h and, finally, the cells were fixed with 4% paraformaldehyde. After DAPI staining, the slides were gel mounted and dried; the images were captured in a fluorescent microscope (Nikon Eclipse 80i). Binding of the quantum dots to the exosomes was also confirmed by transmission electron microscope (TEM). To assess the cytotoxic potential of the IL13QD on the GBM cells, we performed cell proliferation assay with U251 glioma cells. The cells were treated with various concentrations of IL13 conjugated and unconjugated quantum dots in the range 3–25 nM for 48 h. Following the incubation, the cells were treated with Alamar Blue reagent and the cell proliferation was monitored by recording the excitation and emission at 560 nm and 590 nm respectively. The experiment was performed 3 times and the statistical analysis was performed by paired Student's *t*-test.

2.4.2. Exosome isolation

Exosomes were isolated from the condition media of glioma stem cells (designated as T3691 and T387) and from the CSF of the brain tumor patients. The exosomes were isolated by differential centrifugation followed by ultracentrifugation method as described earlier [18,19]. Briefly, for isolating the exosomes from the cells, the cells were cultured to 80% confluence in glioma stem cell culture medium. The condition media was collected 72 h from the time of starting the culture and subjected to differential centrifugation under following sequential spins: 300*g* for 10 min, 16,000*g* for 20 min, filtration through 0.22 μm membrane filter

Download English Version:

https://daneshyari.com/en/article/6449395

Download Persian Version:

https://daneshyari.com/article/6449395

Daneshyari.com