Carbohydrate Research 405 (2015) 93–101

Contents lists available at ScienceDirect

Carbohydrate Research

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

Synthesis and cell-selective antitumor properties of amino acid conjugated tumor-associated carbohydrate antigen-coated gold nanoparticles

Souvik Biswas, Scott H. Medina, Joseph J. Barchi Jr. *

Chemical Biology Laboratory, The Center for Cancer Research, National Cancer Institute at Frederick, Frederick, MD 21702, United States

ARTICLE INFO

Article history:
Received 23 August 2014
Received in revised form 4 November 2014
Accepted 5 November 2014
Available online 3 December 2014

Keywords:
Thomsen Friedenreich
Tumor-associated carbohydrate antigens
Gold nanoparticles
Galectin-3
Cytotoxicity

ABSTRACT

The Thomsen Friedenreich antigen (TF_{ag}) disaccharide is a tumor-associated carbohydrate antigen (TACA) found primarily on carcinoma cells and rarely expressed in normal tissue. The TF_{ag} has been shown to interact with Galectin-3 (Gal-3), one in a family of β-galactoside binding proteins. Galectins have a variety of cellular functions, and Gal-3 has been shown to be the sole galectin with anti-apoptotic activity. We have previously prepared gold nanoparticles (AuNP) coated with the TF_{ag} in various presentations as potential anti-adhesive therapeutic tools or antitumor vaccine platforms. Here we describe the synthesis of TF_{ag}–glycoamino acid conjugates attached to gold nanoparticles through a combined alkane/PEG linker, where the TF_{ag} was attached to either a serine or threonine amino acid. Particles were fully characterized by a host of biophysical techniques, and along with a control particle carrying hydroxyl-terminated linker units, were evaluated in both Gal-3 positive and negative cell lines. We show that the particles bearing the saccharides selectively inhibited tumor cell growth of the Gal-3 positive cells significantly more than the Gal-3 negative cells. In addition, the threonine-attached TF particles were more potent than the serine-attached constructs. These results support the use of AuNP as antitumor therapeutic platforms, targeted against cell lines that express specific lectins that interact with TF_{ag}.

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1. Introduction

Tumor-associated carbohydrate antigens (TACAs) are glycan structures presented primarily on tumor cells and nearly absent on their normal counterparts.[1,2] These unusual structures arise from the aberrant expression of different glycosyltransferases in the transformed phenotype, leading to either extension (N-linked) or truncation (O-linked) of cell-surface glycans.[2,3] As the name implies, these structures are targets of the human immune system (antigens), since they differ from 'self' oligosaccharides. As a result, both active and passive immunotherapeutic approaches against many of these glycan structures have been explored by several groups.[3–13] To date however, no vaccine or antibody therapies targeting TACAs has been translated to the clinic.

TACA expression can be a result of changes in several different steps in the glycoprocessing machinery, including increased/decreased sialylation[14–24] or fucosylation,[25–29] increased N-linked glycan branching, altered O-linked glycolipid (ganglioside) compositions[30–34] and truncated mucin-type O-glycans.[16,35–50] These structures, in part, may modify the physical and chemical properties of the tumor cell, leading to altered cell adhesion and signal transduction, often resulting in enhanced aggressiveness and metastatic potential. Consequently, altered tumor glycosylation is a target of many anticancer therapeutic strategies, including inhibition of glycosyltransferases[51,52] to, in effect, remodel the aberrant glycans toward more 'normal' compositions. Altered tumor glycans may also adversely affect cell adhesion, which is another target of therapeutic intervention.[53]

The Thomsen Friedenreich TACA (herein referred to as TF_{ag}, for 'TF antigen') is a simple, truncated disaccharide, viz., Galβ1–3GalNAc-α-serine/threonine, that is prominently displayed on tumor cells but rarely found on normal tissue.[54] TF_{ag} is an excellent target of anticancer therapeutic intervention, as it acts as a tumor antigen as well as a mediator of metastasis (via lectin-mediated adhesive events) in several solid tumor types.[55–58] Hence, a plethora of approaches have been explored to exploit TF_{ag} as a target for both active[4,5,8] and passive[59–61] immunotherapy; in addition to strategies that inhibit cell adhesion.[57,58,62,63] It is now well established that TF_{ag} engages a specific galectin, Galectin-3 (Gal-3), during the metastatic spread of certain TF_{ag}-bearing tumors, and that this interaction can dictate the aggressiveness of the tumor.[55,63–66]

* Corresponding author. Tel.: +1 301 846 5905; fax: +1 301 846 6033.
E-mail address: barchij@mail.nih.gov (J.J. Barchi).

http://dx.doi.org/10.1016/j.carres.2014.11.002
0008-6215/Published by Elsevier Ltd.

Since the majority of biologically relevant carbohydrate–protein interactions require multivalent binding for enhanced avidity,[67] many of these studies have utilized platforms where the TF_{ag} or a TF_{ag} mimic is displayed in multiple copies for a more potent inhibitory effect.

Our laboratory has been interested in developing new multivalent platforms to display the TF_{ag} in various contexts,[68–71] as potential vaccine constructs or inhibitors of cell adhesion. We have utilized gold nanoparticles (AuNPs) as our 'standard' platform for their ease of synthesis coupled with the ability to attach a variety of molecular families to their surface. In the past several years, the AuNP field has exploded with a variety of constructions that have extremely useful biological/therapeutic utility,[72–74] even one that has found its way to clinical trials.[75,76] Decoration of AuNPs with glycan-based molecules took hold in ~2001 and has advanced our understanding of multivalent carbohydrate–protein interactions.[77,78] TF_{ag}-coated AuNPs from our lab have been prepared both with simple linkers and with the TF_{ag} in the context of mucin-derived glycopeptides; a design that elicits an immune response in mice toward the glycosylated units.[71] Herein we describe the design and synthesis of AuNPs with the TF_{ag} O-linked to the amino acids (serine (Ser) or threonine (Thr)) to which they are commonly presented on cell surface proteins. We hypothesized that cytotoxicity of these particles against tumor cells could be dependent both on the expression of Gal-3 in the cells as well as the amino acid used for conjugation.

2. Results and discussion

2.1. Synthesis of TF_{ag}–amino acid conjugates

In our original design, we synthesized TF_{ag}-coated AuNPs where the anomeric center was α-linked to a simple short polyethylene glycol segment with a terminal thiol for attachment to the nanoparticle surface.[68] In a biological setting, the glycan is attached to a protein through the hydroxyl group of either serine or threonine, and hence this saccharide–amino acid conjugate is often thought of as the actual 'antigenic' structure, not solely the carbohydrate (TACA). We redesigned the synthesis of our TF_{ag}-conjugates to accommodate a single amino acid. This design makes use of the TF_{ag}–glycoamino acid and an appropriate linker for nanoparticle attachment. We chose to synthesize both Ser and Thr conjugated TF_{ag} ligands for coating the nanoparticle's surface, since both our group[79–81] and others[82,83] have shown differential activity and conformational preferences in glycopeptides that are dependent on the glycan attachment to either of these amino acids (Fig. 1A).

In our design, the NH_2 group of both amino acids was N-acetylated to mimic a peptide bond. As we have done previously, we also prepared 'control' AuNPs that bear only the linker unit terminated by a hydroxyl group in place of the glycan (Fig. 1B). The synthesis of the molecules used for AuNP coating is shown in Scheme 1.

Commercially available heptaethylene glycol **1** was mono-protected with one equivalent of 5-bromo-1-pentene affording **2** in 52% yield. Functionalization of the remaining hydroxyl group as the mesylate followed by replacement with azide gave **4** in high yield. Reduction to the amine with lithium aluminum hydride followed by Boc protection and addition of the elements of thioacetic acid across the double bond resulted in compound **6**. Removal of the Boc group with TFA followed by a peptide coupling reaction with peracetylated Fmoc-TF-Serine or Threonine (each either prepared by us[84] or purchased from Sussex Research, Ottawa, Canada) afforded the appropriately-linked TF_{ag} for preparation of gold nanoparticles. We employed coupling conditions that were determined previously by our laboratory to minimize racemization of the α-carbon of the amino acid.[85,86] Final stages for the preparation of the desired AuNP ligand included Fmoc deprotection with piperidine, acetylation of the resulting amino group with acetic anhydride in methanol and Zemplèn deprotection of all O- and S-protected acetates resulting in thiols **10a** and **10b**. For the preparation of the control ligand, compound **2** was processed directly to the thioacetate **11** and hydrolyzed to **12**; this compound was used directly for AuNP synthesis.

2.2. Preparation and characterization AuNPs

Preparations of AuNPs were accomplished by sodium borohydride reduction of gold salts in a methanol solution (Fig. 2). Initial attempts to prepare these materials in water failed to produce uniform particles which were further limited due to aggregation and instability. Using degassed methanol and specific concentrations/ratios of gold salt to carbohydrate thiol conjugate yielded much improved uniformity and stability (see Materials and methods). Hence, compounds **10a**, **10b,** or **12** were employed to prepare

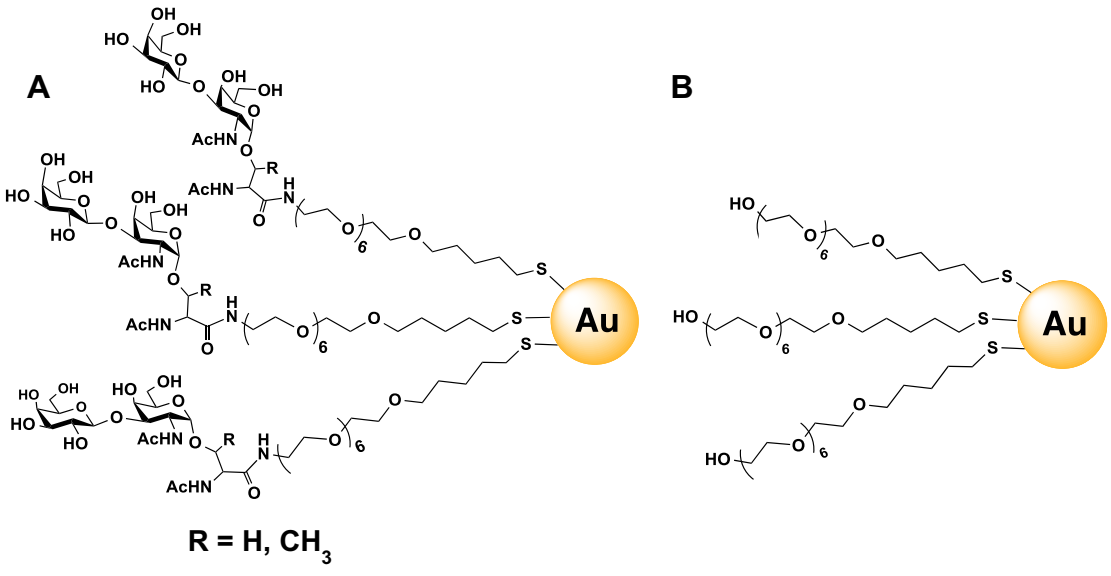

Figure 1. (A) Serine and threonine-conjugated TF_{ag} AuNPs use in this study. (B) Structure of the control linker-conjugated particles.