Bioorganic & Medicinal Chemistry 24 (2016) 4372–4380

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Multiantigenic peptide–polymer conjugates as therapeutic vaccines against cervical cancer

Waleed M. Hussein [a], Tzu-Yu Liu [a], Zhongfan Jia [b], Nigel A. J. McMillan [c], Michael J. Monteiro [b], Istvan Toth [a,d,e], Mariusz Skwarczynski [a,*]

[a] School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, Brisbane, QLD 4072, Australia
[b] Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, QLD 4072, Australia
[c] Cancer Research Centre, Griffith Health Institute and School of Medical Science, Griffith University, Gold Coast, QLD 4222, Australia
[d] School of Pharmacy, The University of Queensland, Brisbane, QLD 4072, Australia
[e] Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD 4072 Australia

ARTICLE INFO

Article history:
Received 11 May 2016
Revised 14 July 2016
Accepted 16 July 2016
Available online 18 July 2016

Keywords:
Peptide-based subunit vaccine
Multiantigenic
Human papillomavirus
Therapeutic cancer vaccine
Polymer–peptide conjugate
Self-adjuvanting
Cervical cancer

ABSTRACT

Immunotherapy is one of the most promising strategies for the treatment of cancer. Human papillomavirus (HPV) is responsible for virtually all cases of cervical cancer. The main purpose of a therapeutic HPV vaccine is to stimulate $CD8^+$ cytotoxic T lymphocytes (CTLs) that can eradicate HPV infected cells. HPV oncoproteins E6 and E7 are continuously expressed and are essential for maintaining the growth of HPV-associated tumor cells. We designed polymer-based multi-antigenic formulations/constructs that were comprised of the E6 and E7 peptide epitopes. We developed an N-terminus-based epitope conjugation to conjugate two unprotected peptides to poly *tert*-butyl acrylate. This method allowed for the incorporation of the two antigens into a polymeric dendrimer in a strictly equimolar ratio. The most effective formulations eliminated tumors in up to 50% of treated mice. Tumor recurrence was not observed up to 3 months post initial challenge.

1. Introduction

Human papilloma viruses (HPVs) are the main cause of cervical cancer.[1] There are currently two prophylactic HPV vaccines, Gardasil and Cervarix, that have been developed and commercialized to the global market.[2] However, they are only recommended for naïve females aged from 9 to 26, and not for women already infected with HPVs.[3] For this reason, a new therapeutic vaccine is required for the treatment of the HPV-infected population.

In the last few decades, peptide-based subunit vaccines emerged as promising prophylactic and/or therapeutic medicines against several infectious diseases.[4] The main components of peptide-based subunit vaccines are the small peptides derived from the protein of a targeted pathogen.[5] In contrast to whole-cell or protein vaccines, vaccine non-redundant peptide components are non-toxic and non-infectious, and significantly lower the risks of allergic and/or autoimmune responses in patients.[6] They have high specificity as their peptide epitopes are purposely designed to recognize certain pathogenic targets. The pure peptides are easily produced under simple and economical methods, and microbe culturing is not required. They are usually water-soluble and stable at room temperature, and do not require special storage conditions. The use of a peptide-based approach in the development of therapeutic anticancer vaccines in contrast to whole oncogenic proteins reduces the risk of vaccine-induced side-effects. However, one of the drawbacks of using peptides is that they require adjuvants as immunostimulant agents to trigger the desired immune responses. Commercially available adjuvants are often weak inducers of anticancer immune responses and/or toxic, and, therefore, new delivery platforms/adjuvants are needed.[6,7]

To be effective, a therapeutic vaccine must be able to induce antitumor T-lymphocyte responses to directly kill cancer cells and, subsequently, to regress tumor growth.[8] The identification of appropriate peptide epitopes capable of initiating effective antitumor T-lymphocyte responses is critical for the design of a therapeutic vaccine.[9] HPV oncoproteins E6 and E7 are continuously expressed and are essential for maintaining the growth of HPV-associated tumor cells. Therefore, $E6_{43–57}$ (QLLRREVYDFAFRDL)[10] and $E7_{44–57}$ (QAEPDRAHYNIVTF) epitopes were chosen for this study. $E7_{44–57}$ contains a $CD4^+$ T helper cell epitope ($E7_{48–54}$, DRAHYNI) and a $CD8^+$ T cell epitope ($E7_{49–57}$, RAHYNIVTF),[11,12]

* Corresponding author. Tel.: +61 7 33469894; fax: +61 7 33654273.
E-mail address: m.skwarczynski@uq.edu.au (M. Skwarczynski).

http://dx.doi.org/10.1016/j.bmc.2016.07.036
0968-0896/

similarly $E6_{43-57}$ also includes both $CD4^+$ and $CD8^+$ epitopes.[13] Recently, we showed that $E7_{44-57}$ conjugated to a polymeric delivery system was able to eradicate E7-expressing tumor cells in immunized mice.[11,12,14] *tert*-Butyl acrylate polymer was chosen as a delivery platform for the vaccine because of its safety profile[15] and ability to serve as a self-adjuvanting moiety to induce both strong humoral and cellular immune responses.[16–18]

In all of the previous challenge experiments, vaccine candidates were used to treat small tumors, as the vaccines were administered 3 days post tumor implantation. However, ideal therapeutic vaccines should also be able to eradicate large, well-established tumors. Unfortunately, the trialed therapeutic vaccine candidates often failed to demonstrate this desired efficacy when used for the treatment of advanced cancer, in both mice models and human clinical trials.[19]

Here, we describe the synthesis of vaccine candidates **1**–**7** (Fig. 1) and the biological evaluation of their ability to eradicate TC-1 tumors from female C57Bl/6 mice. In contrast to previous studies, one of the main purposes of this work is to synthesize and test multiantigenic polymer-based vaccine delivery system, carrying both E6 and E7 protein-based epitopes, against 7 day well-established tumors in challenge experiments.

2. Results and discussion

2.1. Synthesis and characterization of polymer–peptide conjugates

Vaccine candidates **1**–**4** (Fig. 1) were synthesized as described previously.[14] Vaccine candidate **5** was synthesized through CuAAC between the alkyne-functionalized poly(*t*-butyl acrylate) (**8**)[11] and the azido acetic acid derivative of $E6_{43-57}$ epitope (**9**, N_3CH_2CO-QLLRREVYDFAFRDL-NH_2)[20] (Scheme 1).

As the synthesis and evaluation of multiantigenic peptide–polymer carrying both E6 and E7 derived epitopes is one of our main purposes in this work, and the N-terminus conjugation of them is required for their activity,[11] the application of appropriate conjugation strategy is crucial for production of desired multicomponent vaccine candidates. Conjugation of peptides, became a popular approach for the synthesis of chemically engineered biomolecules for various biological applications.[21] Peptides ligation is a smart solution to overcome the obstacles in obtaining large homogeneous peptides with more than 50 amino acids by using solid phase peptide synthesis (SPPS).[22] Many peptide ligation techniques were revealed for the conjugation of two peptides via the binding of amino (N) terminal of one of the peptides to the carboxy (C) terminal of the other one.[23] However, a very few number of research focused on peptide conjugation through their N-terminals. Johnson et al. coupled two copies of an unprotected erythropoietin receptor agonist peptide from their N-terminals by employing an amine-reactive difunctional polyethylene glycol (PEG) molecule, succinimidyl propionate, to form a linear polymer molecule.[24] The presence of an amine group in the side chain of a single lysine within the peptide sequence led to formation of undesired bindings and difficulties in purifications. Szewczuk and co-workers carried out successfully an N-terminal dimerization of a peptide fragment on SPPS by using polyethylene glycols spacer; however, the use of fully protected peptides was required.[25,26] Liskamp and his team were able to conjugate 3 different unprotected cyclic peptides to trialkynes scaffold through copper-catalyzed alkyne–azide cycloaddition (CuAAC) reaction; however, the control of substitution ratio was difficult.[27]

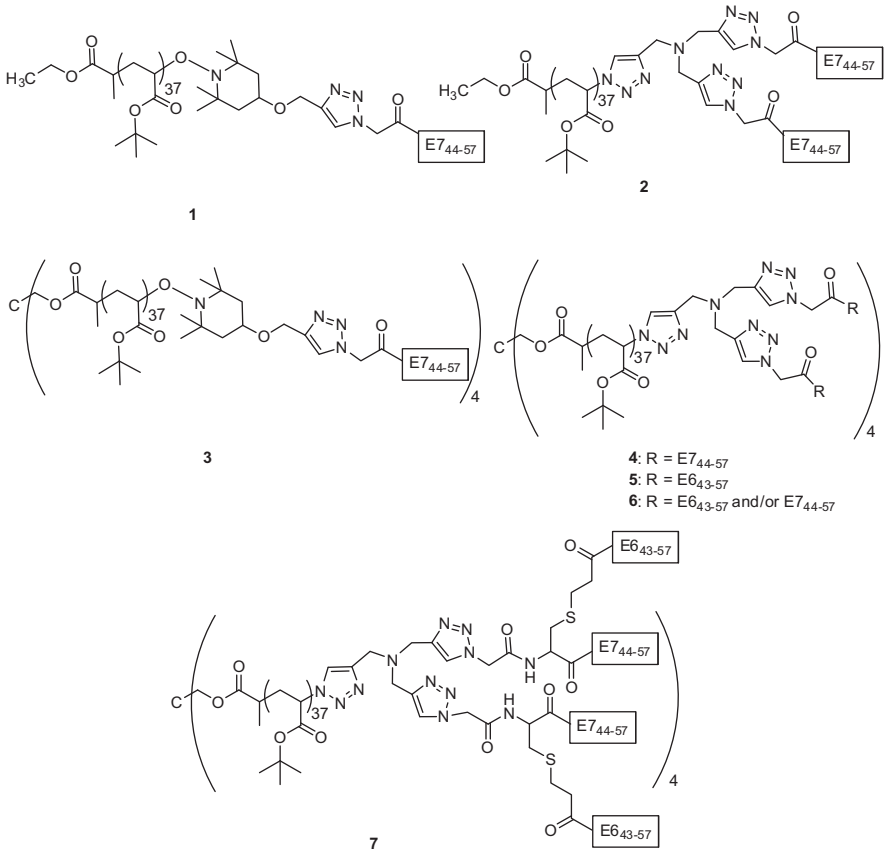

Figure 1. Vaccine candidates **1**–**7**.