Synthesis and Biological Activity of Self-adjuvanting Peptide-based Therapeutic Vaccine Against Cervical Cancer

Tzu-Yu Liu1, Waleed M. Hussein1, Zhongfan Jia2, Saranya Chandrudu1, Nigel A. J. McMillan3, Michael J. Monteiro2, Istvan Toth1,4, and Mariusz Skwarczynski1

1The University of Queensland, School of Chemistry and Molecular Biosciences, Brisbane QLD 4072, Australia; 2The University of Queensland, Australian Institute for Bioengineering and Nanotechnology, Brisbane QLD 4072, Australia; 3Cancer Research Centre, Griffith Health Institute and School of Medical Science, Griffith University, Gold Coast, QLD 4222, Australia; 4The University of Queensland, School of Pharmacy, Brisbane, QLD 4072, Australia.

tzuyu.liu1@uqconnect.edu.au

ABSTRACT SUMMARY

Dendrimers are structurally well-defined, synthetic polymers with sizes and physicochemical properties resembling those of biomolecules such as proteins. Thus, they are promising candidates for peptide-based vaccine delivery platforms. Herein, we conjugated human papillomavirus peptide antigen to polymeric dendritic core. The synthetic pathway to produce polymer-peptide conjugates as macromolecular vaccine candidates against HPV-related cancers was established. These conjugates were able to reduce tumor growth as well as eradicate established E7-expressing TC-1 tumors in mice after a single immunization and without adding any external adjuvant.

INTRODUCTION

Cervical cancer is the second most common cancer in women worldwide and this cancer is caused by high-risk types of human papillomavirus (HPV), most commonly HPV-16. Prophylactic HPV vaccines were commercialized and are clinical effective in preventing HPV infection but do not have a therapeutic effect against established HPV infection. The development of therapeutic vaccines that eliminate HPV infected cells and eradicate established HPV-associated tumors would therefore be beneficial and desirable.1 The aim of this study is to develop a novel therapeutic vaccine strategy based on polymeric dendrimers conjugated to synthetic peptide epitopes derived from HPV-16 E7 protein. The polyacrylate polymer has been selected as the most promising candidate because it has little or no toxicity and the self-assembling and self-adjuvanting ability of polyacrylate amphiphilic dendrimers has been reported.2,3 Three novel peptide epitopes derived from 8Q epitope (E744-62) were employed. All of these epitopes contain a CTL epitope (CD8+), as well as a T-helper cell (CD4+) and a B-cell epitope, and thus all necessary components to stimulate long-term vaccine effectiveness. The three newly designed epitopes, when conjugated to the dendrimer, were able to self-assemble into particles and induced strong immune responses.

EXPERIMENTAL METHODS

We synthesized 4 arms star dendritic structures consisting of polyacrylate cores and three novel peptide epitopes derived from 8Q (E744-62) epitope. The peptide epitopes were synthesized using solid phase peptide synthesis (SPPS), whereas polymeric cores were synthesized by successive atom transfer radical polymerization (ATRP).4 Unprotected peptide epitopes containing an N-terminus azide moiety were conjugated to core structures via copper-catalyzed alkyne-azide 1,3-dipolar cycloaddition “click” reaction. The self-adjuvanting vaccine particles were formed under aqueous conditions and purified by dialysis. The particles were characterized by dynamic light scattering (DLS) and transmission electron microscopy (TEM) to measure particle size, and elemental analysis to determine conjugation efficacy between polymer and peptides.

To test the efficacy of polymer-peptide conjugates as a therapeutic vaccine against established tumors, in vivo tumor treatment...
experiments were performed based on well-established procedures with C57BL/6 mice using TC-1 tumor model.

All data were analyzed using GraphPad Prism 5 software. Results of tumor volumes among all treatments were evaluated by two-way ANOVA test. Kaplan-Meier survival curves for tumor treatment experiments were applied.

RESULTS AND DISCUSSION

We designed polymer-peptide conjugates as therapeutic vaccine candidates against cervical cancer. Three constructs have been synthesized; each contained multiple copies of the same epitope conjugated to the dendrimer. These constructs were self-assembled into particles of different sizes. Modification of immunogenic epitope allowed elimination of undesired disulfide bond-based aggregation/polymerization of the peptide-polymers conjugates. In order to control hydrophilicity of peptide epitope terminal immunologically-redundant pentapeptide in 8Q sequence was scrubbed or modified.

Tumor-bearing mice treated with one of polymer-peptide conjugates showed slower tumor growth over time and similar to that of mice treated with positive control formulation. Additionally, treatment with this conjugate led to significantly better survival compared to treatment with any other immunogens. Chemical conjugation of epitopes with a polymer core was essential to elicit therapeutic effect as dendrimer + 8Q physical mixture failed to induce antitumor immune responses. Three examined conjugates demonstrated different antitumor activity that might be associated with ability of antigen presenting cells to correctly process and display different epitopes.

CONCLUSION

We have established synthetic pathway to produce polymer-peptide conjugates as macromolecular vaccine candidates against HPV-related cancers. Through modification of peptide epitopes, we were able to self-assemble conjugates into particles. The lead conjugate produced therapeutic effect against established tumor without adding any external adjuvant. Noticeably, this polymer-based peptide delivery system produced strong antitumor immune responses after single immunization. Thus, we developed first self-adjuvanting delivery system for therapeutic vaccine against cervical cancer.


REFERENCES


ACKNOWLEDGMENTS

This work was supported by the National Health and Medical Research Council of Australia (NHMRC 1006454).