Formulation and Evaluation of Transdermal Microparticulate Vaccine for Ovarian Cancer

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ABSTRACT SUMMARY
Customized immunotherapeutic strategies may serve as an alternative method to control the recurrence or progression of ovarian cancer. Therefore, in this study, we prepared a vaccine using whole cell lysate of ovarian cancer cell line and characterized its effectiveness in mice model to prevent/retard the ovarian cancer growth via transdermal route.

INTRODUCTION
Ovarian cancer is the fifth most leading cause of cancer related deaths in women in US. It has been observed that the cancer relapses within relatively short periods of time even after the surgery and chemotherapy\textsuperscript{1}. Therefore, immunotherapeutic strategies may serve as an alternative to control the recurrence or progression of ovarian cancer. In this study, we prepared a microparticulate vaccine using whole cell lysate obtained from ID8 murine ovarian cancer cells as this cell line correlates with human ovarian cancer cell lines in terms of various markers and provides a unique model to study ovarian cancer progression and pre-therapeutic trials in mice with intact immune systems\textsuperscript{2}, \textsuperscript{3}. This lysate was entrapped into microparticles made up of a biodegradable and biocompatible polymer matrix. Also, the effect of immuno-stimulatory cytokines such as interleukins IL2, IL12 was studied.

These vaccine microparticles were administered to female C57BL/6 mice by the transdermal route. Serum samples were taken to determine the antigen specific IgG levels to assess the systemic immunity. At the end of the vaccination, animals were challenged with live ovarian tumor cells and the tumor growth was monitored. Mechanistic studies such as natural killer cell activity, CD8+ and CD4+ T-Lymphocyte activity after vaccination were being carried out in order to study the mechanism by which the vaccine works to modulate immune response.

EXPERIMENTAL METHODS
Murine ovarian cancer cell line ID8 was cultured to confluence and treated with hypotonic buffer along with freeze-thaw cycles to obtain whole cell lysate. Microparticulate vaccine was prepared by entrapping whole cell lysate of murine ovarian cancer ID8 cells in a polymer matrix of albumin and eudragit polymers using a Buchi mini spray dryer. These microparticles were administered as prime and boosters via transdermal route with and without interleukins such as IL-2, IL-12 to C57BL/6 female mice. Animals were used as per protocol approved by Institutional Animal Care and Use Committee and Mercer University. Transdermal administration was achieved by using Admin\textsuperscript{®} Pen. Serum was taken before each dosing to check the IgG levels. At the end of the dosing, one group of mice was challenged by injecting ID8 cells subcutaneously and tumor growth rate was monitored. Other groups were used for mechanistic studies. For these studies, different organs such as draining lymph nodes, bone marrow, and spleen of mice were extracted and single cell suspensions were prepared. Flow cytometric analysis for CD8 and CD4 T-cell assays, NK-cell activity were carried out to assess vaccine efficacy.

RESULTS AND DISCUSSION
The total protein concentration of lysate was 1.56 ± 0.5 mg/ml. The particle size range was 800-1500 nm. Zeta potential was 12.54 ± 2.1 mV. The size and zeta obtained was found to be suitable for particle uptake by macrophages. Tumor development was retarded in the vaccinated groups as compared to non-
vaccinated group (p<0.001) (Fig: 1). Interleukin was found to further elevate the immune response (p<0.001). Serum IgG levels were elevated in all vaccinated groups. When serum IgG1 titers (indicative of Th2 response) were analyzed, there was an elevation in titers in mice treated with vaccine with interleukins when compared to non-vaccinated mice. In case of IgG2a titers (indicative of Th1 response), the titers were elevated in case of vaccine with interleukins when compared to placebo (p<0.01) (Fig: 2). CD8+ T-cell, CD4+ T-cell, NK-cells populations were found to be elevated in all vaccinated mice when compared to non-vaccinated group. Moreover, the inclusion of interleukins in the vaccine resulted in even further elevation in this cytotoxic T-cell population (p<0.05) (Fig: 3).

Fig 1: Tumor challenge study

Tumor - Transdermal Vaccination

Weeks after tumor challenge

Fig 2: IgG2a Humoral Response to Vaccine

IgG2a- Transdermal Vaccination

Fig 3: CD8+ T-Cell Response to Vaccine

CONCLUSION

Based upon the vaccine response data, the tumor retardation was found to effective upon transdermal administration. Vaccination using individualized tumor cells may prove to be an efficient treatment for patients in future.

REFERENCES


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