Pathogen mimicking particles in an injectable synthetic-immune-priming center (sIPC) provide efficient immune cell activation and protection in murine tumor models

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ABSTRACT SUMMARY
A multifunctional, cancer vaccine delivery system was developed involving polymeric micro/nanoparticles and an injectable, in situ crosslinking hydrogel to co-deliver tumor antigens and other immunostimulatory molecules to dendritic cells (DCs) for clinically relevant cancer immunotherapy.

INTRODUCTION
Many current cancer therapies result in serious side effects and eventually recurrence. Recently methods that utilize the body’s immune response to eliminate tumor cells have been investigated as a more effective and permanent therapy for cancer. However, conventional immunotherapy concepts are not relevant, mainly due to the weak immunogenicity of tumor antigens. Most efforts to date have failed to elicit a strong immune response in humans due to inability to create multi-modal systems that can simultaneously attract sufficient antigen presenting cells (APCs), ensure efficient delivery of antigens to the antigen presenting cells and modulate the resulting immune response to a specific phenotype suitable for cancer cell elimination.

We are currently testing formulations of a synthetic, injectable immune-priming center (sIPC) consisting of units such as an in situ cross-linking hydrogel carrying APC-attracting chemokines and PLGA microparticles co-loaded with tumor antigens and adjuvants. In addition, we have also begun testing the efficacy of nanoparticles as vaccine carriers as they provide a larger surface area for loading and are taken up by APCs by different mechanisms.

We have demonstrated the versatility of this vaccine delivery platform using protein, peptide, pDNA and tumor lysate based antigens for multiple tumor models. Additionally, the sIPC can simultaneously incorporate different modulatory molecules to aid in immune stimulation, specifically toll-like receptor ligands and siRNA. Ideally, this system will provide a more efficient co-delivery method of tumor antigens and adjuvants to APCs and provide a robust, multi-modal system for effective cancer immunotherapy.

EXPERIMENTAL METHODS
PLGA polymer particles were prepared by single oil/water or double water/oil/water emulsions then were covalently modified with polyethylenimine to form a cationic shell.¹ For surface functionalization, cationic polymer particles were then surface loaded overnight at 4°C with negatively charged antigen and/or with multiple toll-like receptor ligands including siRNA, 1826 CpG, poly (I:C), and poly(U). Immunomodulatory molecules in some cases were also encapsulated into the polymer particles in the internal aqueous phase of the emulsion.

In-situ forming Dextran hydrogels were synthesized as previously described using a Michael addition reaction.² Briefly, vinyl sulfone functionalized Dextran and poly(ethylene glycol) tetrathiol are each suspended in TEA buffer at 10% w/v and then mixed. The mixture remains fluid until heated to 37°C, after which a gel is formed. This provides an in situ forming depot for particles and other molecules.

For in vitro experiments, bone marrow derived dendritic cells (BMDCs) were isolated from the femur and tibia of female BALB/c mice. Cells were cultured for 6 days with growth factor enhanced medium before being treated with varying doses of loaded particles. Particles were incubated with cells for 48 hours before surface marker analysis using flow cytometry.

In vivo studies were conducted by injecting various formulations of the microparticle sIPC with and without Dextran gels, IM or SC. Female Balb/c mice were vaccinated 3 times before or after being challenged with a tumor. Survivability was tracked thereafter.

RESULTS AND DISCUSSION
Micro- and nanoparticles have been successfully loaded with different levels of single immunostimulatory molecules, as well as multiple molecules on the same particle. Approximately 100% of the available molecules are loaded onto the surface of the particle up to a 2% w/w target load. This efficiency decreases as target loading increases, but loading levels of over 20 ug/mg PLGA have been observed. Efficient encapsulation has also been demonstrated for siRNA and tumor antigenic peptides.
In vitro activation studies on BMDCs have produced promising results for microparticle and nanoparticle platforms using a range of molecules. Specifically, addition of toll-like receptor ligands like CpG enhances the expression of surface markers like CD86 and secreted cytokines like IL12p70, indicating DC maturation and activation (Figure 1).

These results are mirrored in mixed leukocyte reaction studies, in which microparticle vaccines induce greater T cell activation and proliferation than soluble forms of the immunomodulatory molecules.

In vivo prophylactic studies also demonstrate the efficacy and versatility of the siPC platform in providing protection against both melanoma and lymphoma tumors using protein and pDNA based antigens, respectively. In Figure 2, it can be appreciated that microparticle vaccines that included immunomodulatory molecules, especially CpG provided protection that lead to significantly improved survival of mice given lymphoma and melanoma tumors, respectively. Additionally, OVA microparticle formulations provided enhanced protection over controls in a therapeutic model as well.

Figure 1: (top) BMDCs stimulated with CpG and poly(I:C) loaded particles result in CD11c+CD86+ cells similar to positive control, LPS. (bottom) Microparticles enhanced BMDC secretion of IL12p70 after being activated with an OVA based protein vaccine and CpG.

Figure 2: (top) Survival of mice vaccinated with microparticles loaded with pDNA, CpG and IL-10 siRNA was enhanced significantly after A20 lymphoma tumor challenge. (bottom) Microparticles loaded with an OVA protein antigen and CpG improved mouse survivability significantly over soluble controls in a melanoma model.

CONCLUSION

PLGA particles serve as an efficient vehicle for delivery of antigen and immunostimulatory molecules to DCs. In conjunction with the in situ forming dextran hydrogel, this system forms an immune depot at the site of injection and can induce a robust cancer targeted immune response.

REFERENCES


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