Local Liposomal Delivery of Anti-CD40 and CpG Stimulates an Anti-Tumor Response while Minimizing Systemic Side Effects

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Introduction. Tumors possess a variety of mechanisms to suppress the immune system in their local environment, enabling them to escape immune eradication. One promising strategy for tumor therapy is to deliver immuno-stimulatory factors that can prime the immune system to combat tumor growth.[1] Monoclonal antibodies against the CD40 receptor on antigen-presenting cells (APCs) have been shown to induce potent anti-tumor immune responses, by increasing tumor antigen presentation, cytokine secretion, and the activation of APCs and NK cells at the tumor. However, the systemic administration of anti-CD40 antibody results in dangerous inflammatory responses in non-target organs such as the liver and lungs. In previous studies, patients in early clinical trials have experienced symptoms of cytokine release syndrome, transient hepatic and hematologic toxicity, and other adverse effects.[2] This motivates the need to localize the bio-distribution of anti-CD40 to the tumor and the tumor-proximal lymph node, in order to optimize an anti-tumor response while minimizing systemic exposure to the agonist. In this study, we propose a strategy to sequester anti-CD40 therapy at the tumor in combination with a CpG oligonucleotide (an immuno-stimulatory TLR9 ligand), via liposomal delivery. We demonstrate that locally restricted immunotherapy can stimulate an effective therapeutic response in tumor-bearing mice, while minimizing adverse systemic effects.

Methods. Liposomes were synthesized from a phospholipid mixture of cholesterol/DOPC/DSPE-PEG/DSPE-PEG-maleimide (35:50:10:5 by mol%). Anti-CD40 antibody was then conjugated to the surface of liposomes via maleimide-thiol reaction (Figure 1A). Incorporation of CpG into antibody-coupled liposomes was performed using a novel synthetic conjugate of phospholipid-PEG_4-CpG, which inserts into the lipid bilayers of liposomes (Figure 1A). Anti-CD40-liposomes and combination (anti-CD40 + CpG) liposomes were extruded to approximate sizes of 100nm and 150nm, respectively, before use. To study the anti-tumor efficacy of these liposome therapies, $5x10^4$ B16F10 melanoma cells were subcutaneously implanted into the right flank of healthy mice. Tumors were allowed to grow for 8 days, followed by 4 doses of therapy, injected intra-tumorally every 2 days. Each dose consisted of 40ug of anti-CD40 (soluble or liposome-conjugated form), with or without 20ug of CpG (soluble or liposome-inserted form). To assess systemic exposure to the immunotherapy, circulating serum levels of anti-CD40 agonist and the pro-inflammatory cytokines TNF-alpha and IL-6 were measured, as well as serum levels of hepatic ALT enzyme, an indication of liver damage.

Results. Maleimide-functionalized liposomes were used to covalently conjugate the anti-CD40 antibody FGK4.5 with an average binding efficiency of 50-60ug of antibody per umol of total phospholipid. *In vitro* release studies showed minimal loss of antibody over 7 days, indicating that the conjugated antibody was stably bound. FACS and histological analysis following intra-tumoral injections of anti-CD40-liposomes showed that the antibody was locally sequestered at the tumor by liposomal delivery *in vivo*. Serum circulating levels of anti-CD40 could be detected in mice that received soluble antibody treatments, but not in those that received liposome-bound antibody. As shown in Figure 1B, locally administered soluble anti-CD40 treatments resulted in increased systemic levels of inflammatory cytokines such as IL-6, as well as the hepatic ALT enzyme (not shown), and gross weight loss in treated animals. In contrast, locally administered liposomal anti-CD40/CpG showed minimal inflammatory systemic side effects. Tumor sizes were also measured to assess the therapeutic efficacy of anti-CD40-liposomes and combination liposomes. Figure 1C shows that anti-CD40-liposomes induced a moderate anti-tumor response, but remained less potent than soluble anti-CD40. However, combination liposomes were highly effective at inhibiting tumor growth, while avoiding the systemic side effects elicited by soluble combination treatment.

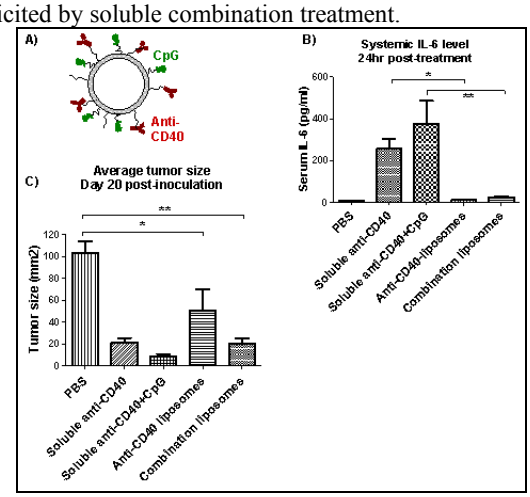

Fig. 1: A) Schematic of combination liposomes. B) serum levels of IL-6 24hr after treatment. *p=0.002, **p=0.02. C) Tumor size at day 20. *p=0.04, **p=0.0001.

Conclusions. We have developed a versatile liposomal system to restrict the bio-distribution of intra-tumorally delivered anti-CD40 and CpG. We demonstrated the potent anti-tumor efficacy of liposomal therapy, while minimizing the adverse side effects of this immunotherapy by dramatically reducing systemic exposure to these broadly stimulatory agonists.

References.

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2. Advani R et al. *J Clin Onc*, **2009**, 27(26), 4371-4377.