Heparin-based IL-12 Complex Coacervate Inhibits B16F10 Melanoma Tumor Progression

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Statement of Purpose: Melanoma is the deadliest type of skin cancer with the fastest increasing incidence rate among all solid tumors. Conventional modes of therapy such as chemotherapy yield a low response rate (<12%)[1] despite various adverse side effects. More recently, immunotherapy has emerged as a viable alternative. Comprised of immune-activating cytokines such as IL-2 and checkpoint inhibitors such as anti PD-1 antibody, current treatment regimens utilize intravenous infusions. Unfortunately, the ineffectiveness of such bolus injections necessitates a high dose, which can then lead to serious side effects and unsustainable economic costs. Consequently, the delivery of such immunotherapeutics via controlled delivery platforms is highly attractive. In particular, complex coacervation presents a biocompatible mode of delivery compared to other methods. In this study, we encapsulate IL-12 in a heparin-based complex coacervation platform. The IL-12 complex coacervate system is tested on an aggressive B16F10 melanoma model and to the best of our knowledge, is the first application of complex coacervate-mediated delivery of proteins for anti-cancer therapeutics.

Methods: A polycation, poly(ethylene aspartate diglyceride) (PEAD) is synthesized as previously described[2]. To prepare complex coacervates, PEAD and heparin are solubilized in 0.9% saline and sterile filtered. The appropriate amount of IL-12 is combined with heparin, after which PEAD is added; the mass ratio of PEAD:heparin is 5.5:1. In-vitro studies were carried out to examine the following: encapsulation and release profile, morphology via cryo-TEM, protection of encapsulated IL-12 from proteases, and bioactivity of releasates via IL-12-mediated release of IFN-γ from mouse splenocytes. In-vivo studies were carried out using 4-6 week old C57BL/6 mice inoculated with bilateral injections of B16F10 cells, and were examined for the following: dose escalation study, survival, tumor growth for primary (site of treatment) and contralateral sites, and immunofluorescence sections of tumors.

Results: IL-12 encapsulation into heparin-based complex coacervates is highly efficient as assessed via ELISA (99.99%); IL-12 is significantly protected from protease-mediated degradation compared to free IL-12. A delayed release of IL-12 is observed for at least 2 weeks, in which the releasate shows increased bioactivity compared to free IL-12, presumably due to heparin-mediated stabilization. Mice inoculated with bilateral injections of B16F10 tumors show a systemic immune response that significantly inhibits tumor growth, even with a single dose of treatment in one tumor site. Natural killer cell infiltration into the tumors is significantly increased at early time points (5 days post-treatment) in mice treated with IL-12 coacervates. At later time points (12 days post-treatment), significant infiltration of CD8 T cells is observed in mice treated with IL-12 coacervates while regulatory T cell presence is minimized.

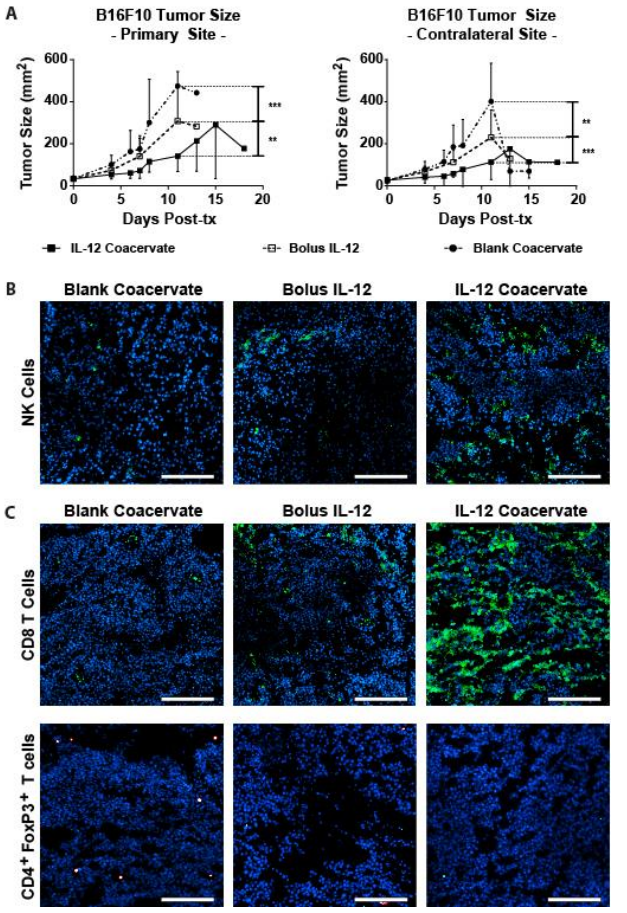

Figure 1. A. Melanoma growth is significantly inhibited for both primary and contralateral sites when treated with IL-12 coacervates (**p<0.01; ***p<0.001). **B.** Natural killer (NK) cell (green) infiltration into tumors is significantly increased in mice treated with IL-12 coacervate (scale bar: 200 μm). **C.** CD8 T cells (green) presence significantly increases in mice treated with IL-12 coacervate, while that of $CD4^+$ $Foxp3^+$ T cells (red and teal colocalized) decreases (scale bar: 200 μm).

Conclusions: A heparin-based complex coacervation platform can be used successfully to deliver IL-12 to significantly inhibit the growth of melanoma in mice.

References:

1. Gogas HJ. Cancer. 2007; 109:455-464
2. Chu H. Biotech. Progress. 2011; 28:257-264