Aerosol delivery of immunotherapy and Flavonoid nanoparticles in murine lung cancer model

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Abstract

Purpose: This project aims to develop a novel treatment for non-small cell lung cancer (NSCLC) using nanotechnology for inhalation drug delivery. A Flavonoid, Hesperetin, bound to PLGA-coated nanoparticles (Hesperetin Nanoparticles, HNPs) and AntiCD40 aiming for localized targeted dosing to reduce the treatment-related systemic toxicity.

Methods: A syngeneic orthotopic murine model of Lung adenoma was generated in wild (+/+) C57/BL6 background mice with similar background LLC1 cell lines. Lung tumor-bearing mice were treated with HNP, antiCD40, or both as an aerosol spray with a control group treated with the solvent (dH_2O) only.

Results: We demonstrate a high intake of the HNP and AntiCD40 by the cancer cells sparing the normal lung tissue. Moreover, the highest percentage of survival rate was observed with the combination aerosol treatment with HNP+AntiCD40 ($p<0.0001$), compared to CD40 alone ($p<0.01$).

Comments: This treatment model will allow us to make the lung cancer treatment method easily available for the mass population without having hazardous radiation treatment in the lung cancer model.

Introduction

Radiation is a common modality for lung cancer treatment. Radiation induces apoptosis, exposing cancer-associated antigens that can be recognized by antigen-presenting cells (APCs) to induce antineoplastic effects by activating cytotoxic T cells. Our prior studies show adding immunoadjuvants like anti-CD40 antibodies with radiation can further activate the APCs. Flavonoids like Hesperetin, an ACE2 receptor agonist, were recently shown to induce apoptosis in lung cancer cells where ACE2 receptors are abundant. Here we developed a novel treatment delivery method in a non-small cell lung cancer (NSCLC) model using inhalation of anti-CD40 and Hesperetin-containing nanoparticles (HNP), to enhance the antitumor effect and to reduce systemic toxicity risks.

Methods and Materials

We prepared Hesperetin Nanoparticles (HNP) using NanoFabTx™ nano-formulation reagent kits (Millipore-Sigma). We developed syngeneic orthotopic murine lung tumors using a luciferase gene transfected LL/2-Luc2 Lewis lung cancer cell line (ATCC) implanted in wild-type C57BL/6 mice (Taconic Mice), enabling time-dependent bioluminescence tumor imaging (BLI). We performed *in-vivo* survival assay analysis after aerosol treatment with HNPs with or without anti-CD40 (abcam).

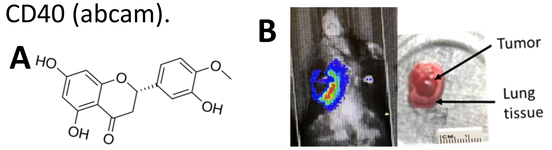

Figure 1. Biochemical structure of Hesperetin (**A**) BLI image of a mouse with orthotopic lung tumor and *ex-vivo* lung tumor (**B**)

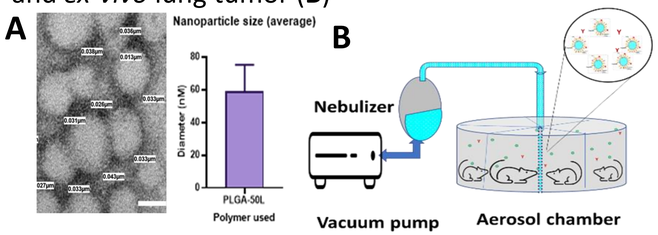

Figure 2. Hesperetin nanoparticles (HNP) were imaged by electron microscopy to analyze the size (30 to 80 nm in diameter) **(A)**. Aerosol administration of HNP and free AntiCD40 was given to mice using a Nebulizer with a vacuum pump connected to a multi-pocket chamber for mice.

Results

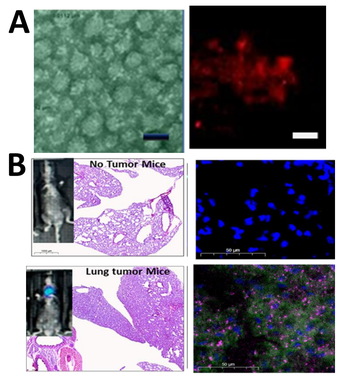

Figure 3. To confirm the aerosol treatment reaches the lung tumor tissue: green polymer NP of the same size along with red fluorescent tagged antiCD40 **(A)** was administered for aerosol delivery. Mice with normal lungs show no uptake by lung epithelial cells and tissue (blue DEPI) **(B)**.

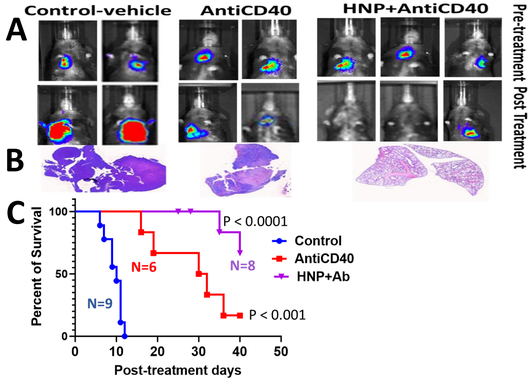

Figure 4. In vivo- analysis of the aerosol treatment of HNP and antiCD40 antibody in mouse model**. (A)**. BLI images and H&E stained histopathological lung images to show the treatment response. (**C**) Kaplan Meier survival graph showing survival percentages and duration in different treatment cohorts.

Discussion

In collaboration with Nanocan Inc we successfully engineered nanoparticles loaded with Hesperetin (HNP), a naturally occurring flavonoid. We engineered a setup that delivers HNP together with the immuno-adjuvant anti-PD1 antibody, as an aerosol inhaled by mice. Our preliminary results in mice demonstrated successful uptake of HNP and anti-CD40 by orthotopically grown lung tumors, but not by normal lung, with a significant increase ($p<0.001$) in survival duration. Key advantages of using this aerosol technique include in-situ delivery of the payload into lung tumor, which overcomes physiological barriers, allowing direct delivery of sufficient payload into the targeted organ, while the standard intravenous delivery results in less than 1-5% drug reaching the tumor.

A detailed analysis is needed to evaluate the application of HNP alone and in combination with other treatment modalities. More studies to define the mechanism of action and the toxicity analysis will also be needed for moving toward a translational outcome.

Conclusions

- We were able to successfully develop an aerosol drug delivery system to administer nanoparticles loaded with drug (HNP) and immunotherapeutic agent (anti-CD40) in a murine lung cancer model.
- We found combining aerosol treatment with anti-CD40+HNP ($p<0.0001$) increased survival compared to untreated controls, or anti-CD40 alone ($p<0.001$). More study is needed to develop an efficient aerosol drug delivery system.

Contact

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