Inhibition of Non-Small Cell Lung Carcinoma (NSCLC) Tumor Growth by Arginine-Albumin Microspheres

Hung-Yen Lee, MS[1, 3], Kamal A. Mohammed Ph.D[1, 2], Zita Burkhalter, BA[1], Eugene P. Goldberg, Ph.D[3] and Najmunnisa Nasreen, Ph.D[1, 2]

[1]Division of Pulmonary, Critical Care & Sleep Medicine, Department of Medicine, [2]NF/SGVHS, Malcom Randall VA Medical Center, University of Florida, P. O. Box 100225, [3]Biomaterials Center, Department of Materials Sciences and Engineering, University of Florida, P. O. Box 116400, Gainesville, FL 32611-6400, USA.

Statement of Purpose: Arginine is a vital amino acid involved in many metabolic processes and biosynthetic pathways that influence tumor growth. Arginine has been reported to inhibit cell growth in variety of cancers including breast, gastric and lung[1-3]. However, higher doses of arginine (>30mM) is required for effective inhibition of tumor cell growth. Therefore the delivery method of arginine to the tumor sites is extremely important. In this study, arginine-albumin microspheres (Arg-BSA-MS) were synthesized to provide a prolonged and localized high concentration of arginine by intratumoral (IT) injection to the tumor site. The inhibitory effect of Arg-BSA-MS for NSCLC (A549) cells was investigated and is reported here.

Methods: Synthesis of hydrophilic Arg-BSA-MS, 50% (w/w) L-arginine and 50% (w/w) BSA, was achieved with reagents dissolved in water and then dispersed with vigorous agitation into an organic solvent to form a MS emulsion. L-arginine loaded BSA-MS was also prepared using a post-loading method. The effects of Arg-BSA-MS to A549 cells (human non-small cell lung adenocarcinoma cell line) were determined *in vitro* by using proliferation (WST-1), wound healing and 3-dimesional tumor growth (Matrigel) assays. The mRNA expression of EphA2 and Slug were measured using real-time PCR.

Results: Arg-BSA-MS was found to be more effective in inhibiting proliferation of the A549 cells than free L-arginine and arginine loaded BSA-MS. Our data demonstrated that the inhibitory effect of Arg-BSA-MS to A549 cells was prolonged. In the wound healing assay, Arg-BSA-MS inhibited cell migration effectively. Wound healing was faster in cells treated with free arginine than with untreated cells. In the tumor growth assay, Arg-BSA-MS inhibited the size and number of tumor colonies in Matrigel. The quantitative real-time PCR results showed that Arg-BSA-MS depressed the expression of EphA2 and Slug in A549 cells within 6 hours, indicative of the inhibition of growth and migration of lung cancer cell.

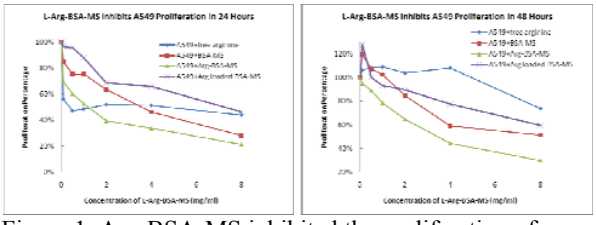

Figure 1. Arg-BSA-MS inhibited the proliferation of A549 cells at 24 and 48 hours.

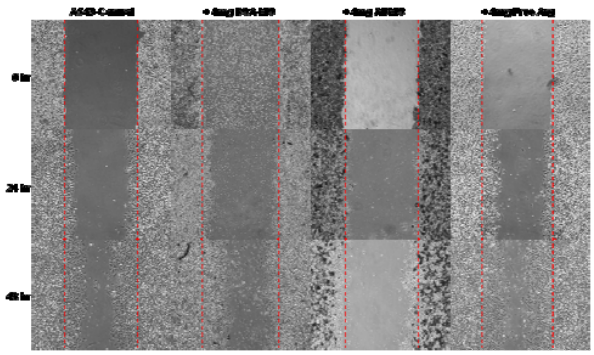

Figure 2. Arg-BSA-MS inhibited the wound healing of A549 at a conentration of 2mg/ml.

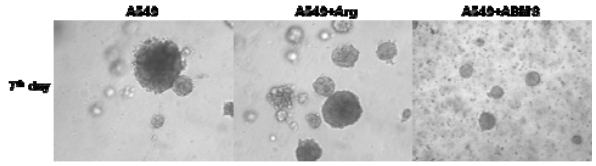

Figure 3. Arg-BSA-MS inhibited the growth of tumor colonies in Matrigel at a concentration of 2mg/ml.

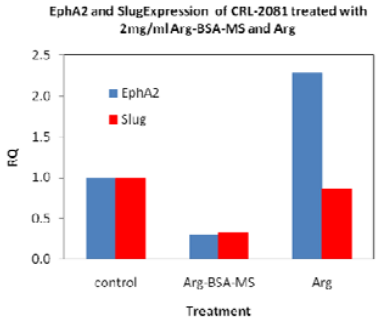

Figure 4. Arg-BSA-MS depressed the expression of EphA2 and Slug in A549 cells.

Conclusions: The findings of this study demonstrates that the L-arginine is an anti-tumor agent and that L-arginine loaded BSA microspheres can be more effective for inhibiting lung cancer cell proliferation than free arginine. Arg-BSA-MS was shown to effectively inhibit cell migration and tumor growth in NSCLC cells.

References: [1.] Cho-Chung YS, Clair T, Bodwin JS, Berghoffer B. Science 1981;214:77-9. [2.] Nanthskumaran S, Brown I, Heys SD, Shcofield AC. Clin Neur 2009;28:65-70. [3.] Shukla J, Thakur VS, Poduval TB. Nitric Oxide-Biol Ch. 2010;22:S83.