Pneumoconiosis Compensation Fund Board research grant: final report Dec 2018

Study title: Targeting polyamine for adjuvant therapy in malignant pleural mesothelioma xenograft models

Abstract

Background: Inhaling asbestos fibers is one of the commonest causes of malignant pleural mesothelioma (MPM). Although the import and use of asbestos have been restricted, the incidence of MPM is still rising due to a long lag time in malignant transformation. In 2004, the US Food and Drug Administration approved a combination of pemetrexed with cisplatin for treatment of unresectable MPM. At the same time, development of novel adjuvant therapeutic options for resected early-stage disease is also urgently needed. From our preliminary data, ornithine decarboxylase is highly expressed in both 211H and H226 MPM xenografts and 4 clinical tumor samples. Upregulation of ODC increases polyamine production and enhances tumor growth. α-difluoromethylornithine (DFMO) is a specific ODC inhibitor. Recent preclinical studies have demonstrated the adjuvant effect of DFMO in colon cancers using xenograft model. However, adjuvant and chemotherapeutic effects of DFMO in MPM have not yet been studied. This study aims to disclose the adjuvant and chemotherapeutic effects of DFMO in MPM xenograft models. The findings from this study will provide scientific foundation for future design of clinical trials of DFMO for adjuvant therapy in early disease for advanced MPM and chemotherapy in

unresectable MPM.

Methods: In adjuvant therapy setting, nude mice were fed with DFMO in drinking water 7 days before subcutaneous inoculation of 200,000 tumor cells (211H (biphasic) or H226 (epithelioid)). In therapeutic setting, 10^7 corresponding cells were injected subcutaneously into the upper back of nude mice, which were randomized for DFMO treatment after established tumor growth. Mice with tumor size >600mm^3 were considered reaching humane endpoint. Spermidine levels, protein expression, cytokines concentrations, and apoptosis were investigated by Dot plot, Western blot, ELISA, and TUNEL assay respectively.

Results: In adjuvant therapy setting, DFMO suppressed tumor growth in both xenografts. DFMO increased median survival from 49.5 days in control arm to 65 days in treatment arm in mice with 211H xenografts ($p = 0.08$), while from 44 days to 120 days in those with H226 xenografts ($p = 0.0002$). In H226 xenograft model, 43% of treated mice have not yet reached humane endpoint, mimicking long-term survival. Upon DFMO treatment, decrease in spermidine level, increase in nitrotyrosine content, and activation of apoptosis were observed in both xenografts. In addition, increase in nitrosocysteine level, intratumoral IL-6, keratinocyte chemoattractant and TNFα, DNA lesion and inhibition of Akt/mTOR pathway were induced by DFMO in H226

xenografts, which may explain higher potency of DFMO in this xenograft.

In therapeutic setting, DFMO also suppressed tumor growth in both xenografts with similar mechanisms though the efficacy was lower than that as an adjuvant therapeutic agent.

Conclusion: DFMO may have a potential role as adjuvant therapy in MPM especially epithelioid mesothelioma.