Abstract

Introduction: Local administration of attenuated mycobacterium species or its product is used as cancer treatment adjuvant to re-boost patient immune response. Macrophage, the primary host and the central effector cells against bacteria, exerts either protection against neoplastic transformation or promotion of growth and metastasis, depending on its phenotype. However, how the attenuated mycobacterium regulates these different macrophage phenotypes to restore their cytotoxicity to cancer cell remains unclear. Thus, we aim to clarify the impact of attenuated mycobacteria stimulation on these macrophage phenotypes.

Methods: Peripheral-blood macrophage collected from lung cancer patients and healthy subjects was stimulated with heat-killed *Mycobacterium tuberculosis* (HKTB) to observe its phenotype alterations. An *in vitro* study utilizing THP1 cells treated with phorbol 12-myristate 13-acetate only (classically activated macrophage, M), or subsequent co-cultured with A549 cells (tumor-educated macrophage, TEM) was developed to investigate the inflammatory profiles and the perturbed mechanism upon HKTB stimulation by flow cytometry, cytokine assay, and proteomics analyses.

Results: HKTB stimulated $CD86^{+}$ pro-inflammatory macrophage population in both healthy subjects and lung cancer patients. The *in vitro* study confirmed this finding, evidenced additionally by increments in TNFα, IL1β, and IFNγ secretions. The stimulated M and TEM showed enhanced but different cytotoxicity to A549 cells. The proteomics analysis of the *in vitro* model revealed that the HKTB induced alterations in lipid metabolism and antigen processing and presentation pathways in M and TEM. In addition, STAT1 and NFkB signallings were upregulated dominantly in M, while mitochondria dysfunction, cell adhesion and migration were enriched in TEM.

Conclusion: Our results suggested that HKTB stimulation regulates distinct intracellular signallings, leading to different degree of pro-inflammatory responses in both M and TEM which improve cytotoxicity to lung cancer cells. Further co-simulation of STAT1 or NFkB-mediated inflammatory responses in TEM is worthy to study for discovering potential immunotherapy adjuvant.

Keywords: macrophage, heat-killed tuberculosis, lung cancer, immune stimulation