The report for the Tang Prize foundation

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2014 Tang Prize Laureate, Tasuku Honjo

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In the study supported by Tang Prize Grant, we have tried to elucidate the mechanisms of unresponsiveness in anti PD-1 Ab therapies, develop the combined therapies to overcome it by targeting immune metabolism [1] [2] [3], and identify biomarkers for predicting the responders [4] [5].

It has been reported that the regulation of energy metabolism, including mitochondrial modulation, could alter immune responses, which would facilitate the control of diseases including cancers. We have found that, during PD-1 blockade therapies, tumor-reactive $CD8^+$ T cells showed an enlarged mitochondrial mass, a higher mitochondrial membrane potential and more mitochondrial superoxide, a major source of mitochondrial ROS (reactive oxygen species), suggesting their mitochondrial function was enhanced. The administration of mitochondrial uncouplers showed a synergistic effect with PD-1 blockade on tumor growth inhibition in a ROS-dependent manner. In this combination therapy, the PGC-1a/PPARs axis, a master regulator of mitochondria activity, was found to be increased. Furthermore, the direct activation of PGC-1α/PPARs axis by bezafibrate, a PPARs agonist which was clinically used as lipid-lowering agent to treat hyperlipidaemia, showed synergistic anti-tumor effects, when combined with anti PD-1 Ab [1]. As for the detailed mechanism of this combination therapy, a significant increase in total

energy metabolism (mitochondrial metabolism and glycolysis) was observed in the T cells of the mice receiving the combination therapy. Activated PGC-1α/PPAR signaling induced high expression of Cptla, a fatty acid oxidation-related gene, and Bcl-2, an apoptosis inhibitory gene, which prevent T cells from activation-induced apoptosis [3]. These systems may convert tumor-reactive T cells, which are supposed to be short-lived, into long-surviving T cells, resulting in an overall increase of T cell numbers and thus enhancing therapeutic efficacy. Following these preclinical studies, combined therapies of nivolumab (anti PD-1 Ab) and bezafibrate have been conducted as an investigator initiated phase I clinical trial.

For the biomarker study, we have collected clinical samples including plasma and PBMC (peripheral blood mononuclear cell) from the anti PD-1 Ab treated patients of non-small cell lung cancer (100< cases), renal cell carcinoma (30< cases), urothelial carcinoma (40< cases) and head and neck cancer (20< cases). In 55 non-small cell lung cancer patients receiving nivolumab, we measured 247 metabolites in the plasma and 52 cell cellular markers in the PBMC. The four metabolites derived from microbiome (hippuric acid), fatty acid oxidation (butyrylcarnitine) and redox (cystine and glutathione disulfide) provided high response probability (AUC=0.91). Similarly, a combination of four T cell markers, those related to mitochondrial activation (PGC-1 expression and reactive oxygen species), and the frequencies of $CD8^+$ $PD\text{-}1^{high}$ and $CD4^+$ T cells demonstrated even higher prediction value (AUC=0.96). Among the pool of all selected markers, the four T cell markers were exclusively selected as the highest predictive combination probably due to their linkage to the above mentioned metabolite. These results suggest the combination of these metabolites and cellular markers can be a quite

valuable biomarker for predicting the efficacy of anti PD-1 Ab [5].

PD-1, PD-L1, and CTLA-4, targets of current immune checkpoint blockade therapies, are released from cell surface as soluble forms. Given the extensive involvement of these immune-regulating molecules in immune reactions and immune-related diseases, detailed investigations of the soluble forms of PD-1, PD-L1, and CTLA-4 can offer insight into new biomarker targets. In particular, high serum concentrations of the soluble PD-1 (sPD-1), PD-L1 (sPD-L1), and CTLA-4 (sCTLA-4) have been associated with several autoimmune diseases. However, several technological issues have limited their clinical application as biomarkers. For instance, enzyme-linked immunosorbent assay (ELISA) is typically used to measure the levels of sPD-1, sPD-L1, and sCTLA-4, but is known to suffer from poor precision and reproducibility due to the manual procedures of experiments. To overcome these limitations, we developed an automated measurement system for sPD-1, sPD-L1, and sCTLA-4 based on a chemiluminescent enzyme immunoassay (HISCL system). The system is fully automated, providing high reproducibility. Application of this system to plasma of patients with several types of tumors demonstrated that sPD-1, sPD-L1, and sCTLA-4 levels were increased compared to those of healthy controls and varied among tumor types. The sensitivity and detection range were sufficient for evaluating plasma concentrations before and after the surgical ablation of cancers. Therefore, our newly developed system shows potential for accurate detection of soluble PD-1, PD-L1, and CTLA-4 levels in the clinical practice [4].

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1. Chamoto K, Chowdhury PS, Kumar A, et al. Mitochondrial activation chemicals synergize with surface receptor PD-1 blockade for T cell-dependent antitumor activity.

Proceedings of the National Academy of Sciences 2017; **114**(5): E761-E70.

2. Chowdhury PS, Chamoto K, Honjo T. Combination therapy strategies for improving PD-1 blockade efficacy: a new era in cancer immunotherapy. *Journal of Internal Medicine* 2018; **283**(2): 110-20.

3. Chowdhury PS, Chamoto K, Kumar A, Honjo T. PPAR-Induced Fatty Acid Oxidation in T Cells Increases the Number of Tumor-Reactive CD8+ T Cells and Facilitates Anti-PD-1 Therapy. *Cancer Immunology Research* 2018; **6**(11): 1375-87.

4. Goto M, Chamoto K, Higuchi K, et al. Analytical performance of a new automated chemiluminescent magnetic immunoassays for soluble PD-1, PD-L1, and CTLA-4 in human plasma. *Scientific Reports* 2019; **9**(1).

5. Hatae R, Chamoto K, Kim YH, et al. Combination of host immune metabolic biomarkers for the PD-1 blockade cancer immunotherapy. *JCI Insight* 2020; **5**(2).