HKU Med LKS Faculty of Medicine
Department of Medicine
香港大學內科學系

Establishment of peripheral blood lymphocytes and lung tumor organoids co-culture model

Jiashuang Yang[1], Hoi-Hin Kwok[1], Junyang Deng[1], Nerissa Chui-Mei Lee[1], David Chi-Leung Lam[1*]
[1]Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

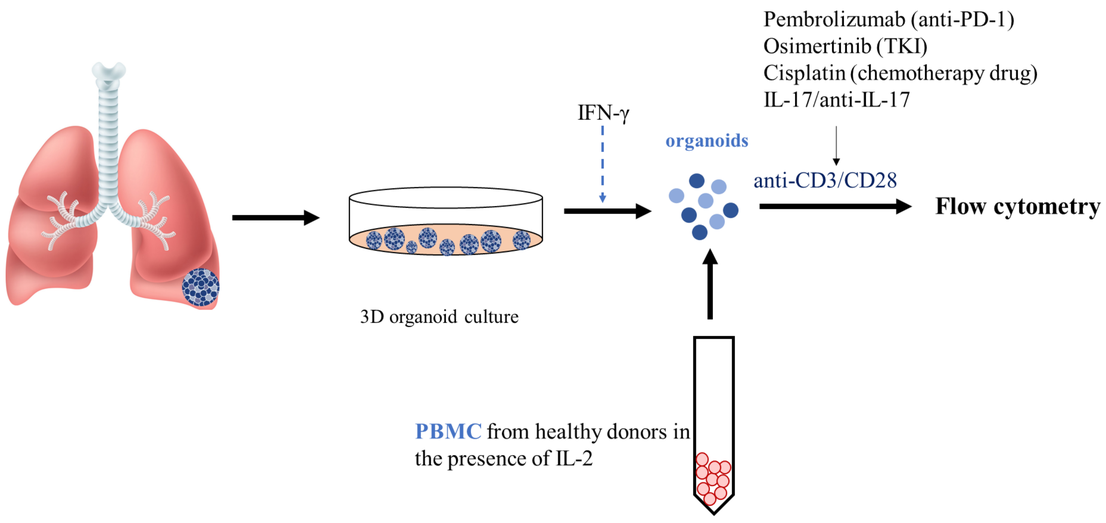
Abstract

Non-small cell lung cancer (NSCLC) is one of most common cancers worldwide, which leads to high mortality. Epidermal growth factor receptor (*EGFR*) has normal functions in cell proliferation, differentiation and migration. Mutations and overexpression of *EGFR* gene were associated with occurrence of malignant diseases and tumor progression in NSCLC. Tyrosine kinase inhibitors (TKIs) is the first line therapy for *EGFR* mutation positive NSCLC patients. Immune checkpoint inhibitors have been widely used to restrain tumor progression and rescue immunosuppression in advanced stage lung cancer as well. However, targeting PD-1/PD-L1 pathway fails to boost immunosurveillance of immune cells in *EGFR* mutation positive NSCLC patients. In our study, we demonstrated PD-L1 is inducible by IFN-γ in NSCLC *EGFR* wildtype (WT)/mutant (MT) tumor organoids. Perforin and granzyme B are released by CD8+ T cells when PBMCs were cocultured with *EGFR* MT tumor organoids. Perforin is released by CD8+ T cells, with also increased surface expression of CD107a, when PBMCs were cocultured with *EGFR* WT tumor organoids. The combination treatment of Osimertinib with pembrolizumab (anti-PD-1) enhances the cytotoxic capacity of PBMCs, compared with Osimertinib alone on *EGFR*-MT NSCLC tumors. Pembrolizumab (anti-PD-1) can increase cytotoxicity of PBMCs, but this cytotoxicity appeared to be increased with anti-IL-17 to *EGFR*-WT NSCLC. Herein, a new peripheral blood lymphocytes and lung tumor organoids co-culture model will be established to improve a better understanding to NSCLC tumor microenvironment and enhance the therapeutic effect of immunotherapy on *EGFR* mutant NSCLC cell lines.

Introduction

Clinically, the therapeutic effect of immunotherapy shows the weak immune response on *EGFR*-MT lung cancer patients. Distinct therapies are used on NSCLC *EGFR*-MT and *EGFR*-WT patients due to the different interactions between NSCLC *EGFR*-MT or *EGFR*-WT tumors and immune cells in tumor microenvironment. However, the downstream of immune response and its mechanism of NSCLC *EGFR*-MT/WT tumors are still unclear. Our purpose is to find out a novel platform to improve immunotherapy and clarify the downstream mechanism on NSCLC *EGFR*-MT tumors.

Methodology



NSCLC organoids were derived from malignant pleural effusion and resected tumor tissue and they were continually cultured to three-dimension tumor organoids. PBMCs were obtained from heathy individuals. Before coculture, PBMCs were cultured in the presence of IL-2. With the stimulation of anti-CD3/CD28 and IL-2, PBMCs were cocultured with tumor organoids to illustrate T cell response and tumor cytotoxicity. The cell surface and intracellular markers were detected by flow cytometry.

Results

1. Induction of PD-L1 expression on tumor organoids by IFN-γ.

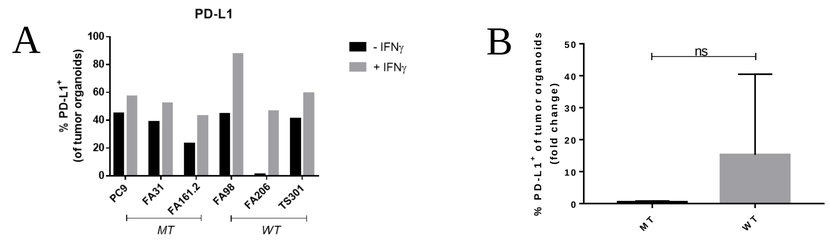


Figure 1. (A) PD-L1 expression level on *EGFR*-MT (PC9, FA31 and FA161.2) and *EGFR*-WT (FA98, FA206 and TS301) tumors after the simulation of IFN-γ for 24 hours was measured by flow cytometry. (B) Fold change of PD-L1 expression on *EGFR*-WT tumors is higher than PD-L1 expression on *EGFR*-MT tumors after IFN-γ stimulation. *P<0.05, **P<0.01.

2. Upregulation of cytotoxic markers of $CD8^+$ T cells upon *EGFR*-MT/WT tumor organoids cocultured with PBMCs.

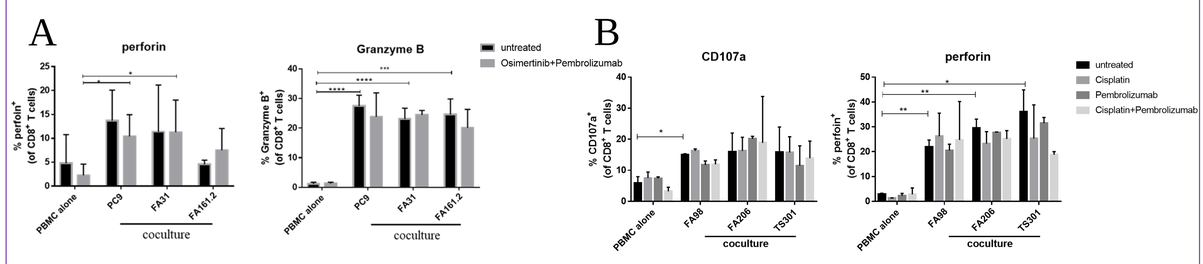


Figure 2. (A) Perforin and granzyme B released by $CD8^+$ T cells increased after coculturing PBMCs with *EGFR*-MT tumors (PC9, FA31 and FA161.2) for 12 hours with Osimertinib plus pembrolizumab treatment. (B) Perforin release and CD107a expression by $CD8^+$ T cells increased after coculturing PBMCs with *EGFR*-WT tumors (FA98, FA206 and TS301) for 12 hours with cisplatin alone, pembrolizumab alone, or cisplatin plus pembrolizumab treatment. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

3. PD-1 expression on $CD8^+$ T cells is blocked by pembrolizumab treatment in *EGFR*-MT/WT NSCLC tumor organoids.

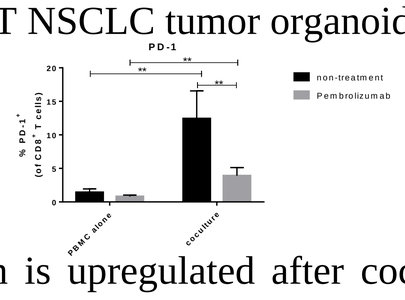


Figure 3. PD-1 expression is upregulated after coculturing PBMCs and NSCLC tumors. PD-1 expression reduced after pembrolizumab treatment in coculture condition. **P<0.01.

4. The effect of Osimertinib and combination of Osimertinib with Pembrolizumab treatment on *EGFR*-MT NSCLC tumor organoids. The effect of Pembrolizumab and combination of Pembrolizumab and anti-IL-17 treatment on *EGFR*-WT NSCLC tumor organoids.

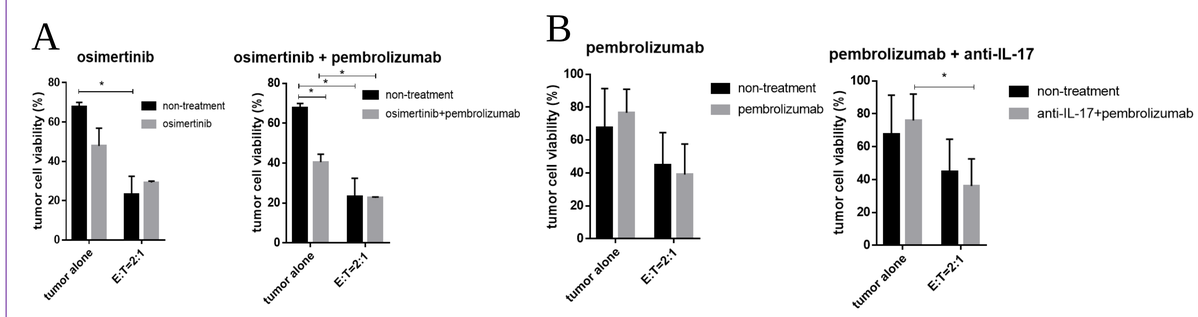


Figure 4. (A) The combination treatment of Osimertinib with pembrolizumab can enhance the cytotoxic capacity of PBMCs on *EGFR*-MT NSCLC tumors. （B）Pembrolizumab can increase cytotoxicity of PBMCs to *EGFR*-WT NSCLC tumors, but this cytotoxicity appeared to be increased with anti-IL-17. *P<0.05.

Conclusion

Our study demonstrated a potential model to explore the interaction between cytotoxic T cells and tumor organoids that would be useful as a model for in vitro screening for therapeutic drugs and relevant immune-modulatory mechanisms.

*correspondence: David Chi-Leung Lam, dcllam@hku.hk