Immune Cell Function and Effect of Immune Checkpoint Inhibitor in Freshly Dispersed Human Tumors

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Introduction

In human cancer tissues, tumor cells coexist with various immune cells, and their interaction creates a complex tumor microenvironment (TME). Understanding immune responses in the TME is important for elucidating immunosuppressive mechanisms mediated by cancer cells and for developing novel immunotherapies.

In this study, fresh human cancer tissues were enzymatically dissociated into single-cell suspensions, and the composition of tumor-infiltrating immune cells was analyzed by flow cytometry. In addition, we evaluated T-cell responsiveness to various stimuli and further examined the effects of immune checkpoint inhibitor on T-cell responses.

Highlight of this study

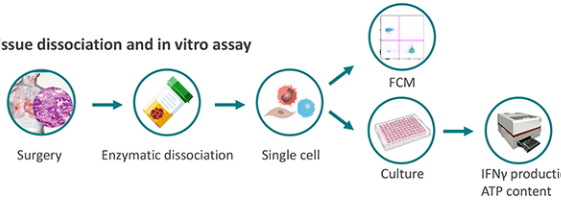
- Fresh human tissue acquisition system was established at Axcelead DDP.
- FCM analysis revealed that immunosuppressive subsets, including PD-1^{high} CD4/CD8 T cells, Tregs and PD-L1^{high} M2 macrophages, were present in the cancer tissue.
- Our in vitro system for evaluating T cell responses in fleshly isolated tumor-derived cells may support the development of novel cancer immunotherapy agents.

Material & Methods

Fresh human tissue acquisition workflow

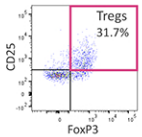
Approved by all relevant ethics committees, and informed consent was obtained.

Tissue dissociation and in vitro assay

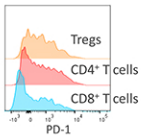


Results

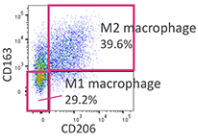
High frequency of Tregs



PD-1 expression on T cells



High frequency of M2 macrophages



PD-L1 expression on Macrophages

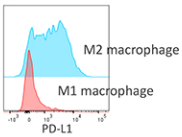


Figure 1 Tumor tissues contain immunosuppressive cells such as PD-1^{high} CD4/CD8 T cells, Tregs, and M2 macrophages.
Tumor tissue (from 82 years old, female, treatment-naïve colon cancer) was enzymatically dissociated into single-cell suspensions, stained with a panel of flow cytometry antibodies and a viability dye, and subsequently analyzed by flow cytometry.

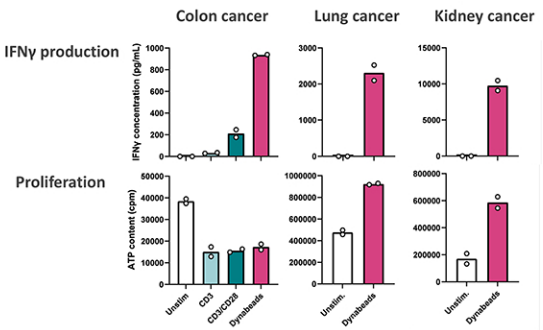


Figure 2 Freshly dispersed tumor-derived cells produced IFNγ upon T-cell stimulation, whereas proliferation was not consistently induced.
Tumor tissues (3 donors: colon, lung or kidney cancer, all treatment-naïve) was enzymatically dissociated into single-cell suspensions and seeded into 96-well plates. Cells were stimulated with anti-CD3 antibody, anti-CD3/CD28 antibodies, or Dynabeads. IFNγ concentration in culture supernatants was measured by ELISA, and intracellular ATP levels were measured by CellTiter-Glo.

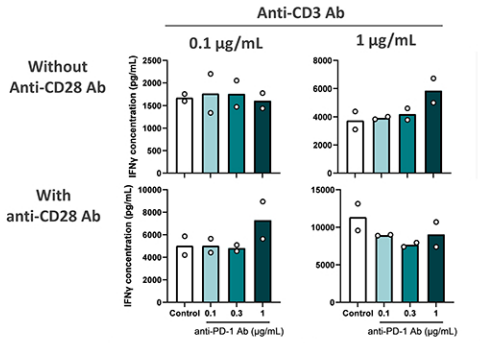


Figure 3 Anti-PD-1 antibody enhanced IFNγ production in a dispersed tumor-derived cells.
Tumor tissue (from 75 years old, female, treatment-naïve kidney cancer) was enzymatically dissociated into single-cell suspensions and seeded into 96-well plates. Cells were treated with anti-PD-1 antibody and stimulated with anti-CD3 antibody, either in the presence or absence of anti-CD28 antibody. IFNγ concentrations in culture supernatants were measured by ELISA.

Conclusion

PD-1^{high} CD4^{+}/CD8^{+} T cells, Tregs, and PD-L1^{high} M2 macrophages were identified in tumor tissues, indicating the presence of an immunosuppressive microenvironment. T-cell activation was assessed using IFNγ production as a functional readout, and anti-PD-1 antibody enhanced IFNγ production. These findings suggest that IFNγ production serves as a functional marker of TIL activation and that the in vitro assay system may mimic some characteristic features of the human TME.

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