Anti-PD-1 scFv producing recombinant *Bifidobacterium* exerts antitumor effect in a larger fraction of the treated mice comparing to full length anti-PD-1 antibody

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Abstract #A23

ABSTRACT

The anti-PD-1 therapy has improved therapeutic outcomes of patients in multiple cancer types. However, the therapy has demonstrated clinical benefits in only a small fraction of patients. The reason of the limited response in clinical practice is not fully understood.

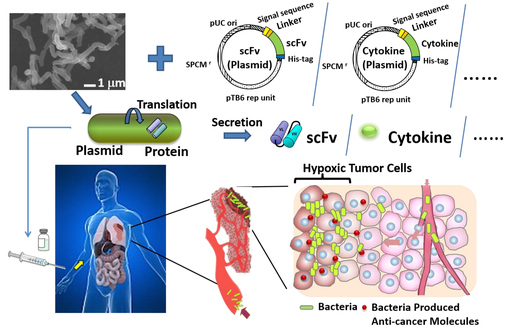
In an aim to improve anti-cancer drug delivery and potency, we have been developing *in situ* Delivery and Production System (*i*-DPS) by modifying a non-pathogenic anaerobic bacterium, *Bifidobacterium*, which localizes and proliferates only in the hypoxic environment like solid tumors after intravenous administration, produces anticancer proteins, enzymes or other pharmacologically active molecules selectively at the tumor site. Here we present anti-human PD-1 scFv producing *i*-DPS in cancer immunotherapy, which could be specifically delivered to and amplified only at the hypoxic sites of solid tumors. A series of *in vitro* assays has been performed to confirm the stable expression and secretion of anti-human PD-1 scFv by recombinant *Bifidobacterium*, the binding inhibition of PD-1/PD-L1 interaction and elevated IFN gamma production in mixed lymphocyte culture by anti-human PD-1 scFv secreted from recombinant *Bifidobacterium*.

Anti-murine PD-1 scFv producing *Bifidobacterium* as surrogate systemically administered to the syngeneic mice model demonstrated significant tumor growth inhibition. Of particular interest, the suppression of tumor growth was observed in a larger fraction of the treated mice while the control anti-PD-1 antibody showed the effect on only a few mice. The analysis of tumor infiltrating lymphocytes and myeloid cells will be presented as well. Taken together, *i*-DPS for anti-PD-1 antibody provides a new promising immune-therapeutic modality to target hypoxic solid tumors and also provides a unique insight for antibody drug delivery in cancer immunotherapy.

BACKGROUND

How does our platform technology work?

***In situ* Delivery and Production System (*i*-DPS)**



***Bifidobacterium longum*: The host cell for *i*-DPS**

- Derived from human intestinal gut flora
- Nonpathogenic obligate anaerobe
- Alive and grow at hypoxic environment
- Dead at the normoxic conditions (blood, organs)

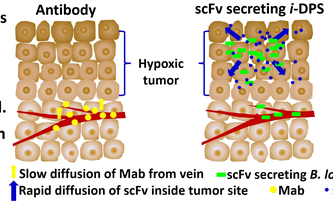
***B. longum* is a good drug delivery carrier because:**

- Selectively colonize and proliferate in hypoxic tumor after systemic injection *in vivo*
- Be engineered with genes coding various biologically active molecules in plasmid (antibodies, cytokines, etc.)

Roles of *i*-DPS in immunotherapy

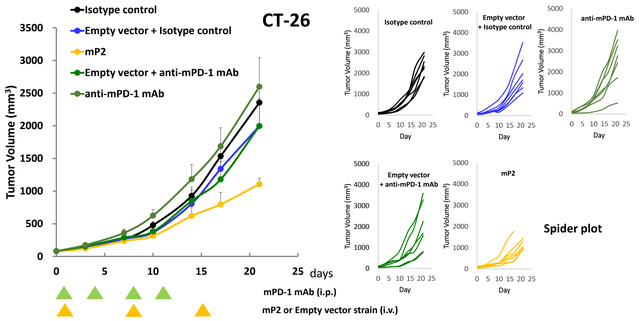
To produce scFv against immune checkpoints inside the tumor

- Immune checkpoint blockade leads anti-cancer drug development. However, off-target effects and severe immune related adverse events such as autoimmune diseases still need to be addressed.
- *i*-DPS offers unique delivery system to allow immune checkpoint blockers be generated inside the tumor.

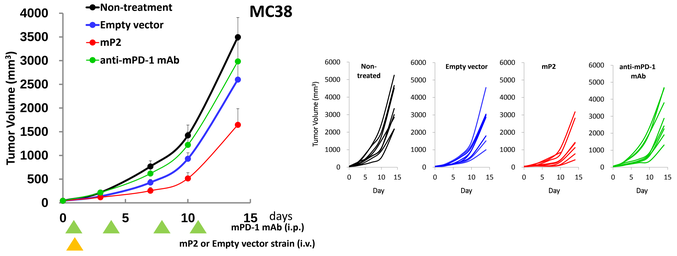


RESULTS

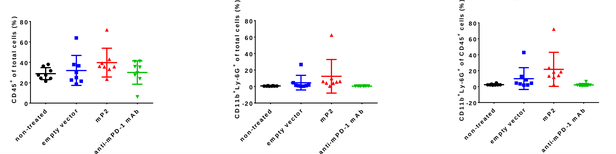
Anti-tumor effects of anti-mPD-1 scFv *i*-DPS (mP2) and anti-mCTLA4 scFv *i*-DPS (mC1F) against CT26 in Balb/c mice and its TIL profiling



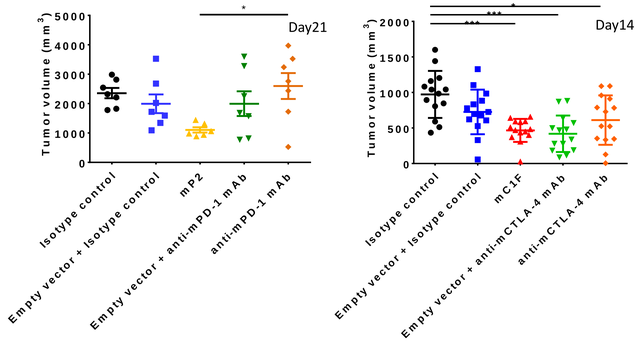
Anti-tumor effects of anti-mPD-1 scFv *i*-DPS (mP2) against MC38 in C57bl/6 mice and its TIL profiling



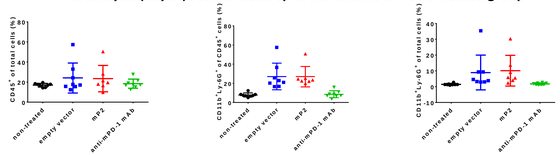
TIL analysis (day 7) reveals neutrophil increments in *i*-DPS treated group



Large portion of mice responds to both anti-mPD-1 scFv *i*-DPS and anti-mCTLA4 scFv *i*-DPS therapy



TIL analysis (day14) reveals neutrophil increments in *i*-DPS treated group



Conclusion & Perspectives

- We have demonstrated the tumor suppression effects of anti-murine PD-1 scFv *i*-DPS both in syngeneic CT26 and MC38 mice models. It is noteworthy that the tumor suppression activities were shown in a large fraction of the treated mice whereas relatively small fraction of tumor bearing mice responds to the full-length anti-mPD-1 Ab treatment. In addition, we also observed the similar anti-tumor activities with anti-mCTLA4 scFv *i*-DPS. Altogether, the above observation suggested that the *i*-DPS therapy enhanced tumor specificity and efficacy, which is expected therapeutic potential to enhance patient response rate in the clinic.
- FACS profiling of immune biomarkers revealed an increment of neutrophils in the tumors treated with anti-mPD-1 scFv *i*-DPS and the empty vector *i*-DPS. These neutrophils may activate immune reaction, which subsequently assists scFv to attack tumor. To elucidate how *i*-DPS improves the distribution of the ICP blockers and how Bifidobacterium cooperates immune system inside tumor would be worthwhile for the better understanding of clinical study results obtained in the future.

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