Cancer immunotherapy with agonistic anti-4-1BB scFv producing and secreting Bifidobacterium in syngeneic mouse model

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ABSTRACT

Agonistic antibodies against immune checkpoint molecules, such as 4-1BB, reportedly demonstrate antitumor effects at the early phase, whereas immune-related adverse events hamper further development. A single chain variable fragment (scFv), which is expected to have better penetration into tissues, is clinically being investigated, however, poor distribution into tumor tissues due to rapid clearance is a serious issue. In order to improve drug delivery and decrease adverse events, we have developed in situ Delivery and Production System (i-DPS) by genetically engineering a non-pathogenic anaerobic Bifidobacterium. We previously reported that the modified Bifidobacteria localized and proliferated only in the hypoxic area of the tumor engrafted in the mouse post i.v. injection, thus producing anti-tumor molecules persistently and selectively at the tumor site (AAMC2010).

Here we present i-DPS with agonistic anti-murine 4-1BB scFv (anti-4-1BB scFv). We created a recombinant Bifidobacterium producing and secreting anti-m4-1BB scFv (BB-2 strain). The anti-m4-1BB scFv-secreting IFN-γ production by 71% over the control group (PBS) in the mouse spleenocytes activated by anti-CD3 antibody in vitro. Anti-tumor effects of Bifidobacteria producing the anti-m4-1BB scFv were demonstrated in the syngeneic model of CT-26 in BALB/c mouse (61.7% tumor growth inhibition (TGI) at 1 x 10^7 cfu/mouse, i.v. on day 14). We further evaluated the anti-tumor effects of i-DPS for anti-m4-1BB scFv 1 x 10^6 cfu, as a combination with anti-mPD-1 antibody (i.p.), and confirmed that the combination therapy significantly suppressed the tumor growth on day 21 (TGI: combination, 60.7%; anti-m4-1BB scFv, 38.8%; anti-mPD-1 mAb, 8.9%). In conclusion, i-DPS for anti-4-1BB scFv will provide a new promising modality for the immunotherapy targeting hypoxic solid tumors.

BACKGROUND

How does our platform technology work?

In situ Delivery and Production System (i-DPS)

*Produce CD enzyme inside the tumor to convert 5-Fc to 5-FU
Tumor selective localization of APS001F

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)

The phase 1 clinical study for APS001F is ongoing in the US.

RESULTS

1. Anti-m4-1BB scFv secreting Bifidobacterium
   - Structure of bacterial expression vector for anti-m4-1BB scFv
   - Purified anti-m4-1BB scFv from BB-2 strain culture supernatant

2. Anti-m4-1BB scFv increased the IFN-γ production in mouse spleenocytes activated by anti-CD3 antibody
   - Mouse spleenocytes were cultured with anti-m4-1BB scFv in the presence of anti-CD3 antibody for 72 hours. Culture supernatants were collected and mIFN-γ concentration was measured by ELISA.

3. Anti-tumor effect of anti-m4-1BB secreting Bifidobacterium (BB-2) combined with anti-mPD-1 antibody against CT26 mouse tumor model

CONCLUSION

- We have established anti-m4-1BB scFv secreting Bifidobacterium and confirmed secretion of anti-m4-1BB scFv from the BB-2 strain.
- Anti-m4-1BB scFv secreting Bifidobacterium enhanced the IFN-γ production of anti-CD3 antibody activated mouse spleenocytes. Also, binding activity of anti-m4-1BB scFv was confirmed (data not shown).
- Anti-m4-1BB scFv producing Bifidobacterium monotherapy showed tumor suppression activity, whereas combination with anti-mPD-1 mAb notably strengthened tumor suppression.
- All above evidence suggest anti-m4-1BB scFv secreting Bifidobacterium is a worth-trying combination candidate for anti-PD-1 antibody