Evaluation of Genetic Modified Salmonella Typhimurium as a Targeted Therapy for Neuroblastoma: Comparison of Response of Orthotopic Mouse Models with Different Immunological Background

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Backgrounds
- Neuroblastoma is an aggressive paediatric tumor with poor prognosis. Neuroblastoma cancer stem cells thrive in hypoxic microenvironment of the tumor core and they are resistant to conventional treatment.
- Genetic engineered anaerobic Salmonella YB1/Sal-YB1 only proliferates under hypoxic environment for it can only synthesize diaminopimelic acid (DAP), an essential component of the cell membrane, under anaerobic condition.
- We investigated the therapeutic effectiveness and bio-safety of Sal-YB1 to both immunodeficient mice and environment.

Materials and Methods
- The adenral orthotopic xenograft was induced by human neuroblastoma (SK-N-LP-Leu) cell line.
- Nude mice (deficient in T cells) and NOD-SCID mice (deficient in T, B & NK cells) were used in orthotopic mouse model.
- In vivo imaging and Salmonella colony forming units were used to investigate the therapeutic effectiveness and biosafety.
- Tumor tissues were assessed by western blot, Elisa assay and histological staining.

Results

Fig. 1 Sal-YB1 could suppress neuroblastoma growth in both nude and NOD-SCID mice. Tumor growth without Sal-YB1 treatment was assessed by (A) in vivo imaging system; and (B) tumor size measurement. (C) ROH change rate was significantly between nude and NOD-SCID mice (P < 0.05). (D) Tumor regression rate was 70% in nude mice and 30% in NOD-SCID mouse (P < 0.05). (E-F) In both nude and NOD-SCID mice, the mice of treated group regained body weight from Day 5.

Fig. 2 Sal-YB1 could cause necrosis in neuroblastoma xenografts of both nude and NOD-SCID mice. Tumor with YB1-treated & untreated group was evaluated by (A) Flow cytometry (FCM), (B) Hematoxylin-Eosin (H&E) and (C) Immuno-histochemistry (IHC) staining respectively. Sal-YB1 treated tumor tissues showed more necrosis and Salmonella could be detected within the tumor mass. Original magnification of B & C was 200x.

Fig. 3 Sal-YB1 could be detected in normal solid tissue and stool. Salmonella Cfu test on (A) tumor & solid tissues, (B) stool, Stool negative mouse could detect Salmonella in blood, bile and brain.

Fig. 4 Potential signaling pathway in Salmonella treated neuroblastoma. Abbreviation: TLR4, Toll-like receptor 4; IRAK, Interleukin 1 receptor-associated kinase; TAK1, TGF-β-activated kinase 1; IKK, IκB kinase; NF-κB, Nuclear factor kappa B; TNFα, tumor necrosis factor, alpha-induced protein 6; TSG6, TNF-stimulated gene 6 protein; Casp, Caspase.

Fig. 5 Sal-YB1 could cascade the Caspases apoptosis signalling pathway. (A) JC-1 could be identified in neuroblastoma after either Sal-YB1 direct treatment or co-culture with Sal-YB1 pre-treated macrophage supernatant. (B, C) YB1-treated mouse tumor tissue showed high expression of Caspase3 and TSG6 through Flow cytometry and Western blotting. (D) Caspase3, 3, 8, 9 and TSG6 could be increased in Sal-YB1 treated tumor tissues by Western blot.

Fig. 6 TNFα & pan-Casp. inhibitors could reverse the anti-neuroblastoma effect by macrophage. Pan-Casp. & anti-TNFα significantly increased the viability of neuroblastoma in macrophage supernatant treated group (P < 0.001).

Fig. 7 Sal-YB1 could up-regulate TLR4 expression and down-regulate the expression of IRAK and IκBα in tumor tissues. (A) TLR4 Flow Cytometry. (B) IRAK (P < 0.05) and IκBα (P < 0.001) Western blotting showed significant decrease in YB1-treated group.

Fig. 8 Sal-YB1 could enhance the anti-neuroblastoma effect of macrophage via TNFα. Elisa assay quantified Sal-YB1 targeting effect on both (A) mouse tumor tissue and (B) human macrophage. (C) The viability of neuroblastoma was decreased when co-cultured with 50% supernatant of the YB1 pre-treated macrophages (P < 0.05) but it could be rescued by TNFα inhibitor (P < 0.01).

Conclusions
- Our approach provides a new paradigm in targeting cancer cells residing within the hypoxia microenvironment.
- Even in mice with severe immunodeficient background such as nude or NOD-SCID, our Sal-YB1 could induce tumor necrosis without causing severe infection.
- The macrophage may play a critical role in Sal-YB1 directed therapy.

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