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 adrenal 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 imagining 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

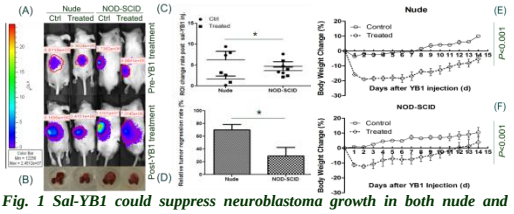

Fig. 1 Sal-YB1 could suppress neuroblastoma growth in both nude and NOD-SCID mice. *Tumor growth with/without Sal-YB1 treatment was assessed by (A) in vivo imaging system; and (B) tumor size measurement. (C) ROI change rate was significantly different between nude and NOD-SCID mice ($P < 0.05$). (D) 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 3.*

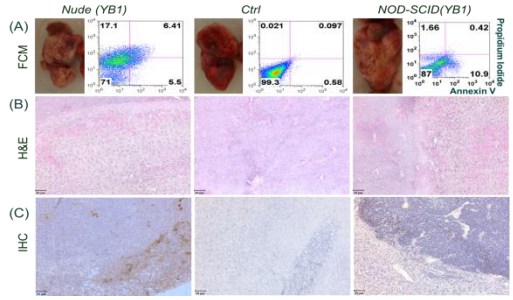

Fig. 2 Sal-YB1 could cause necrosis in neuroblastoma xenografts of both nude and NOD-SCID mice. *Tumor with YB1-treated & -untreated group was evaluated by (A) Flow cytometry (FCM), (B) Hematoxylin-Eosin (H&E) and (C) Immuno-histochemistry (IHC) staining respectively. Sal-YB1 treated tumor tissue showed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

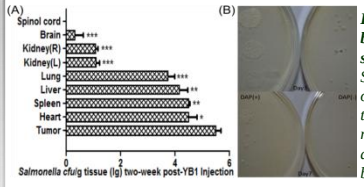

Fig. 3 Sal-YB1 could be detected in normal solid tissue and stool. *Salmonella CFU test on (A) tumor & solid tissues, (B) stool,. Stool negative mouse could detect Salmonella in blood, bile and brain.*

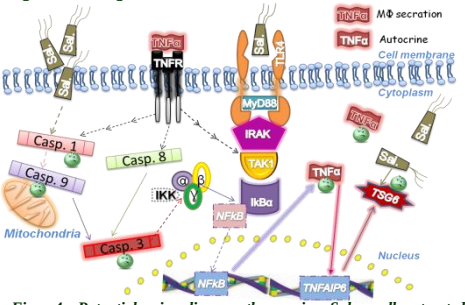

Fig. 4 Potential signaling pathway in Salmonella treated neuroblastoma. *Abbreviation: TLR4, Toll-like receptor 4; IRAK, Interleukin 1 receptor-associated kinase; TAK1, TGF-β-activated kinase 1; IKK, IκB kinase;* ***NF-kB, Nuclear factor kappa B;*** *TNFAIP6, tumor necrosis factor, alpha-induced protein 6; TSG6, TNF-stimulated gene 6 protein; Casp., Caspase.*

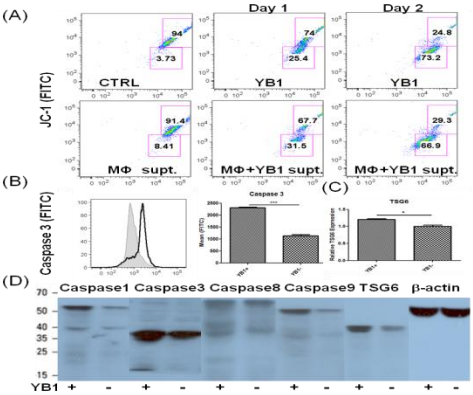

Fig. 5 Sal-YB1 could cascade the Caspases apoptosis signalling pathway. *(A) JC-1 could be identified in neuroblastoma after either Sal-YB1 direct treatment or coculture with Sal-YB1 pre-treated macrophage supernatant. (B, C) YB1-treated mouse tumor tissue showed high expression of Caspase3 and TSG6 through Flow cytometry and Western blotting. (D) Caspase1, 3, 8, 9 and TSG6 could be increased in Sal-YB1 treated tumor tissues by Western blot.*

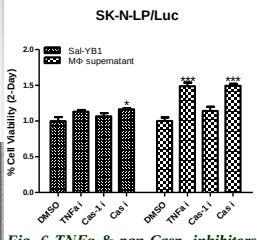

Fig. 6 TNFα & pan-Casp. inhibitors could reverse the anti-neuroblastoma effect by macrophage. *Pan-Casp. & anti-TNFα significantly increased the viability of neuroblstoma in macrophage supernatant treated group ($P < 0.001$).*

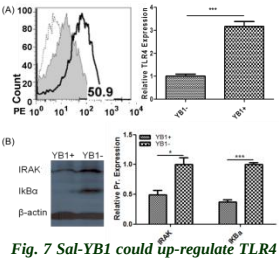

Fig. 7 Sal-YB1 could up-regulate TLR4 expression and down-regulate the expression of IRAK and IkBα in tumor tissues. *(A) TLR4 Flow Cytometry. (B) IRAK ($P < 0.05$) and IkBα ($P < 0.001$) Western blotting showed significant decrease in YB1-treated group.*

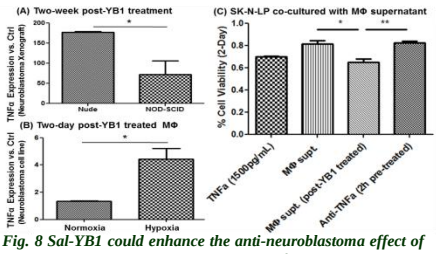

Fig. 8 Sal-YB1 could enhance the anti-neuroblastoma effect of macrophage via TNFα. *Elisa assay quantified Sal-YB1 targeting effect on both (A) mouse tumor tissue and (B) human macrophage. (C) The viability of neuroblastoma was decreased when co-cultured with 50% supernatant of the YB1 pre-treated macrophages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

Acknowledgements

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