Engineered bacteria----novel cancer therapy

Abstract

Attenuated *Salmonella typhimurium* VNP20099 (VNP) has unique characteristics of high tumor targeting and low toxicity. It can highly colonize central parts of tumor tissues. Our project aims to decrease the influence of the bacteria on normal tissues by enhancing the bacteria’s ability of tumor targeting through modifying the amino acid metabolism pathways. We also use VNP as a vector to express exogenous genes with anaerobic promoters in the upper stream to further improve the effect of tumor targeting. Thus, the bacteria could be used as an efficient method of tumor treatment.

Hypoxia-induced gene expression

Here, we engineered *S. typhimurium* strain VNP20099 to carry vectors driven by nirB promoter that express our therapeutic gene encoding tumor-related apoptosis-inducing ligand (TRAIL).

Bacterial lysates were prepared from VNP20099 carrying constructed pTRAIL grown in anaerobic or aerobic jars and immunoblotting assays were then conducted using anti-TRAIL antibodies. These findings indicated that genes we need for Salmonella-mediated tumor targeting therapy could be effectively expressed in VNP under the control of a hypoxia-induced nirB promoter.

Immunoblotting studies further showed that TRAIL was effectively expressed in VNP20099 growing under hypoxia which carries the plasmid constructing pTRAIL.

Conclusion

Both the results of screened strains and the experiments of PnirB in vitro and in vivo are exciting. Based on VNP, we reconstructed the strains and had a 50-fold increase in tumor targeting of Salmonella. In the meantime, we also proved that anaerobic promoter nirB can help Salmonella express dozens of times in anaerobic areas than in aerobic areas. Moreover, in the mouse model, VNP expressed specifically in the core areas of tumors as expected. Just imagine a combination of an improved strain and a highly effective promoter, they will eventually become a highly tumor-targeting gene expression system.

Optimization of the Salmonella

We found CFU (Colony Forming Units) of auxotrophic strains 5 is three times than that of VNP in tumor. The ratio of number of bacteria in tumor tissues to that in liver on the 4th day shows that after knocking out related genes of the metabolism of synthesizing certain amino acid, the ability of tumor targeting was highly enhanced, nearly 50 times compared to that of VNP.