Targeted inactivation of Salmonella Agona metabolic genes by group II introns and in vivo assessment of pathogenicity and anti-tumour activity in mouse model

ABSTRACT

The fight against cancer has been a never-ending battle. Limitations of conventional therapies include lack of selectivity, poor penetration and highly toxic to the host. Using genetically modified bacteria as a tumour therapy agent has gained the interest of scientist from the past few decades. Low virulence and highly tolerability of Salmonella spp. in animals and humans make it as the most studied pathogen with regards to anti-tumour therapy. The present study aims to construct a genetically modified S. Agona auxotroph as an anti-tumour agent. LeuB and ArgD metabolic genes in ΔSopBΔSopD double knockout S. Agona were successfully knocked out using a Targetron gene knockout system. The knockout was confirmed by colony PCR and the strains were characterized in vitro and in vivo. The knockout of metabolic genes causes significant growth defect in M9 minimal media. Quadruple knockout ΔSopBΔSopDΔLeuBΔArgD (BDLA) exhibited lowest virulence among all of the strains in all parameters including bacterial load, immunity profile and histopathology studies. In vivo anti-tumour study on colorectal tumour bearing-BALB/c mice revealed that all strains of S. Agona were able to suppress the growth of the large solid tumour as compared with negative control and ΔLeuBΔArgD (LA) and BDLA auxotroph showed better efficacy. Interestingly, higher level of tumour growth suppression was noticed in large tumour. However, multiple administration of bacteria dosage did not increase the tumour suppression efficacy. In this study, the virulence of BDLA knockout strain was slightly reduced and tumour growth suppression efficacy was successfully enhanced, which provide a valuable starting point for the development of S. Agona as anti-tumour agent.

Keyword : Anti-tumour therapy; ArgD gene; Attenuated Salmonella Agona; Colorectal cancer; Group II intron; LeuB gene.