TITLE: CONSTRUCTION AND EVALUATION OF THE ANTINEOPLASTIC ACTIVITY OF ATTENUATED MUTANTS OF *Salmonella* Typhimurium PRODUCING VIOLACEIN

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ABSTRACT:
Salmonella enterica Typhimurium (*S.* Typhimurium) is the most studied bacterium in bacterial-mediated cancer therapy. Our group has demonstrated the antitumor potential of some mutants for genes encoding nucleoid-associated proteins, including the double mutant Δ*ihf*AΔ*ihf*B genes. The treatment with the double mutant in combination with the natural pigment violacein demonstrated antitumor effect in bladder cancer model (unpublished results); however, it exhibited some toxic effects. This study aimed to construct and evaluate the antineoplastic activity of attenuated *S*. Typhimurium violacein-producing mutants.We constructed two triple mutants using the Lambda red system: *S.* Typhimurium Δ*ihf*AΔ*ihf*BΔ*pmi*and *S.* Typhimurium Δ*ihf*AΔ*pmi*Δ*asd*. The virulence of the mutants was evaluated in the *Galleria mellonella* model and compared with the parental strain *S.* Typhimurium 14028. 10^4 CFU were inoculated in last left proleg and survival was monitored for 96 hours. To engineer the attenuated mutants of *S.* Typhimurium to express violacein, we used the plasmid pBAT*vioABCDE*, which loads the violacein operon. pBAT*vioABCDE* was transformed by electroporation into the mutants. The growth of the producing and non-producing violacein mutants was evaluated in LB for 12 hours. The invasiveness and intracellular survival of violacein-producing and non-producing mutants was evaluated by the gentamicin assay. The viability of melanoma and bladder cancer cells treated with violacein-producing and non-producing mutants were evaluated by the MTT assay. In the *in vivo* model 100% of the larvae inoculated with *S.* Typhimurium 14028 died after 24 hours; 50% of the larvae inoculated with the two mutants survived after 96 hours, suggesting that the mutations contribute to the attenuation of *S*. Typhimurium. Colonies transformed with pBAT*vioABCDE* showed a purple coloration after 24 hours, indicating the production of violacein. We observed that the production of violacein significantly decreased the growth rate of the mutants, as well as their capacity for invasion and survival. We also observed that both the violacein-producing and non-producing mutants had the ability to decrease the viability of melanoma and bladder cancer cells by more than 40%, however, the decrease in cell viability was significantly greater in cells treated with violacein-producing mutants. Our results suggest that violacein-producing *S.* Typhimurium have the potential to be used as immunotherapeutics in the treatment of cancer.

Keywords: immunotherapy, *Salmonella* Typhimurium, bacterial-mediated cancer therapy, attenuation, violacein.

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