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Production of a novel recombinant polypeptide from *Serratia marcescens* for cancer therapy

José García Suárez, Zeila Santana Vázquez, Lourdes Zumalacárregui de Cárdenas, Diamilé González Roche and **María del C Abrahantes Pérez**
Center for Genetic Engineering and Biotechnology, Cuba

Cancer has become one of the leading causes of death worldwide, so the interest to discover new drugs with antitumor effect has increased during the last years. In the Center for Genetic Engineering and Biotechnology, Havana, Cuba, a novel polypeptide derived from tumor regression mediated by infection with *Serratia marcescens* was isolated. This molecule was produced by the bacteria as a 50 kDa protein (P50) secreted into the culture medium. It reached biological activity after degradation and exposing its active site located at the carboxyl extreme. Natural degradation occurred after incubation at 37oC, during 12 hours in a non- specific way. Therefore, the reproducibility to obtain a pure preparation was not possible. The analysis of the amino acid sequence of the P50 showed an only methionine at the 231 position, which is cleaved by the carboxylic extreme in the presence of cyanuric bromide. The use of a potential pathogenic microorganism (*S. marcescens*) and a toxic compound (BrCN) made this procedure risky. Therefore a synthetic gen provided by GenArt Company was cloned in a bacterial plasmid and transformed in several *E. coli* strains. As part of this work the Escherichia coli W3110 was selected as the host strain. Fermentation conditions were set at 5 L bench scale and scaled up 50 L working volume. The recombinant protein was expressed insoluble intracellularly as inclusion bodies. After mechanical disruption, followed by non-chromatographic and chromatographic purification steps, an Active Pharmaceutical Ingredient, able to be used as a parenteral product was obtained. *In vitro* and *in vivo* studies were brought about, showing security and a selective inhibitory effect on several tumor cell lines. The Clinical Trial Authorization application was presented to the Cuban Drug Regulatory Authority which approved Phase I Clinical trial. This study is ongoing.

jose.garcia@cigb.edu.cu