CONSTRUCTION OF A MAMMALIAN EXPRESSION VECTOR FOR GOMESIN, AN ANTIMICROBIAL PEPTIDE FROM THE SPIDER ACANTHOSCURRIA GOMESIANA

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In the last two decades, antimicrobial peptides were isolated from different organisms ranging from prokaryotes to humans. These peptides are characterized to participate in the innate immune response as a rapid first line defense against infection. Gomesin, an antimicrobial peptide isolated from hemocytes of the tarantula spider *Acanthoscurria gomesiana*, exhibits broad activity against the growth of Gram-positive and –negative bacterias, filamentous fungi, yeast and eukariotic parasites. In addition, its structural properties confer high stability to the molecule, suggesting that gomesin is a promising candidate for the development of new drugs that act as antibiotics or against new targets. The goal of this study was to construct a plasmid vector to express gomesin in mammalian cells. The active portion of gomesin coding sequence was amplified by PCR from the pGEM-Gom plasmid with primers containing *Bam* HI and *Bgl* II restriction sites (primer 1: 5'-ggatccatgCAGTGCCGTAGGTTGTG-3'; primer 2: 5'-ATTGCCGCGGAAGTGagatct -3'). It was initially cloned in the PCR 2.1 TOPO plasmid (Invitrogen, Carlsbad, USA) for further insertion of four copies in tandem of the coding sequence in the pSP72 vector (Promega, Madison,USA). The first copy was introduced into *Bam* HI and *Bgl* II restriction sites within the pSP72 and the other three copies were inserted into *Bgl* II restriction site only, originating the pSP72/DG4 vector. A secretory signal sequence from hemoglobin was amplified by PCR from the pMAC/PS plasmid with primers containing *Xho* I and *Bam* HI restriction sites (primer 1: 5'-accctcgagATGACATTGAACATGCTG-3'; primer 2: 3´-ATAGTTCCACACGTAACAcctaggca-5´) and cloned into pSP72/DG4 in order to direct the peptide to extracellular spaces. After these steps the signal sequence and the four copies of gomesin were amplified by PCR (primer 1: 3´-ATAGTTCCACACGTAACACCTAGGCA-5'; primer 2: 5'-GAGACCTTCAGATCGCAC-3') and inserted into the pcDNA3.1/V5-His-TOPO plasmid (Invitrogen, Carlsbad, USA) under the control of the CMV promoter. The pcDNA3.1/DG4 vector has been used to transform CHO and MBDK cells lines in order to study the expression of gomesin in mamallian cells.

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