53rd Annual Meeting of SBBq

53rd Annual Meeting of the Brazilian Society for Biochemistry and Molecular Biology (SBBq)
Águas de Lindóia, SP, Brazil - May 18th to 21st, 2024

SBBq

G.41- Intracellularly Produced Amblyomin-X is Targeted by the Proteasome in Melanoma

Posolo-Cinel, V.D.2,1, DeOcesano-Pereira, C.2, Fessel, M.R.3,1, Chudzinski-Tavassi, A.M.3,1
1Biologia Molecular, Universidade Federal de São Paulo (São Paulo, Brasil), 2CENTD, Instituto Butantan (São Paulo, Brasil), 3LDI, Instituto Butantan (São Paulo, Brasil)

Cancer, characterized by uncontrolled cell growth, remains a leading cause of global mortality. Biodiversity-derived proteins have been explored as a promising approach in cancer treatment. Amblyomin-X (AMB), a recombinant kunitz-like protein derived from Amblyomma sculptum tick, has shown selective antitumor activity in tumor cells. However, challenges in large-scale production using bacterial systems persist due to its complex structure. This study aimed to evaluate the intracellular production of AMB in human melanoma cells. Assess the intracellular production of Amblyomin-X in melanoma cells and investigate its degradation pathway. Plasmids encoding AMB for subcellular targeting (none or mitochondria) were synthesized. Transfection in SKMEL28 melanoma cells was followed by MTT assay to assess cytotoxicity. RT-PCR and western blot were conducted to detect AMB expression. To investigate the role of lysine residues in AMB stability, site-directed mutagenesis was performed on lysine residues predicted to have higher probability of ubiquitination according to bioinformatics analysis. The proteasome inhibitor MG132 was used to elucidate the degradation pathway. Transfection efficiency reached approximately 50% in SKMEL28 cells. While bacterially-produced AMB led to a significant reduction in cell viability, intracellularly produced AMB did not. Although intracellular AMB production was confirmed at the mRNA level in SKMEL28 cells, its presence at the protein level was not detected. Site-directed mutagenesis of lysine residues failed to stabilize AMB, suggesting an alternative degradation pathway. Treatment with MG132 resulted in the detection of both cytoplasmic and mitochondrial AMB by western blot, indicating proteasome-mediated degradation independent of ubiquitination. Our findings demonstrate the intracellular production of AMB by melanoma cells and its targeted degradation via the proteasome pathway. Despite efforts to stabilize AMB, lysine mutations did not prevent degradation, highlighting the complexity of its degradation mechanism. Our next step involves the expression of AMB in melanoma cells with a secretory signal to protect the molecule from proteasome-mediated degradation. Keywords: Amblyomin-X, Melanoma, Proteasome

G.42- Evaluation of the antimicrobian potential of dimeric peptides analogous to the peptide (p-BthTX-I)2K

Salvador, L.C.1, Nunes, L.O.C.2, Bicho, G.F.H.1, Argentin, M.N.3, Candido, V.T.3, Camargo, I.L.B.C.3, Cilli, E.M.1, Santos-Filho, N.A.1
1Bioquímica e Química Orgânica , Instituto de Química de Araraquara - UESP (São Paulo, Brasil), 2Análises Clínicas, Faculdade de Ciências Farmacêuticas de Araraquara - UNESP (São Paulo, Brasil), 3Física e Ciência Interdisciplinar , Instituto de Física de São Carlos - USP (São Paulo, Brasil)

The excessive use of drugs in health and agriculture contributes significantly to the growth of bacterial resistance. In this sense, alternative molecules are being studied for this purpose and antimicrobial peptides are considered as promising. This project studies analogues of the peptide (p-BThTX-I)2K, a lysine dimer with higher activity than its corresponding monomer. The peptides in this work, derived from the C-terminal region of the BaMTx and CoaTx-II myotoxins found in Bothrops atrox and Crotalus oreganus abissos snakes. Furthermore, were evaluated the antimicrobial potential along with toxicity in red blood cells and stability in blood serum. Peptides were synthesized by solid phase peptide synthesis (SPFS) and purified by high performance liquid chromatography (HPLC). Minimum Bactericidal Concentration and Minimum Inhibitory Concentration tests were carried out to determine antimicrobial activity. Stability was determined by degradation test in blood serum and toxicity against red blood cells was obtained by hemolytic activity. Dimeric peptides LC2202 and LC2203 were properly synthesized and purified. Minimum bactericidal concentration and minimum inhibitory concentration tests indicated that both peptides have activity against gram-positive and gram-negative bacteria. Times of 0 h, 1 h, 4 h, 12 h and 24 h were considered for analyzing the stability of the peptides in serum. The peptide LC2202 was stable after 24 hours, while the LC2203 was completely degraded in 4 hours, generating degradation products. Hemolytic activity was carried out in serial dilutions of 512 to 1 mg/mL and both peptides did not cause hemolysis at all the concentrations tested. Peptides showed promising antimicrobial activity and satisfactory stability for future studies, as they also showed no toxicity to human cells. In addition, dimerization by lysine (K) proved to be advantageous due to the greater facility of synthesis and good expression of the results mentioned.
Keywords: Antimicrobial peptides, Dimeric peptides, Bacterial resistance