Proteomic analysis of the effects of Bothrops jararaca venom in murine fibroblasts.

Isabel de Souza Carvalho[1], Ana Luiza Teixeira Silva[1], Alison Felipe Alencar Chaves[1], Solange Maria de Toledo Serrano[1]

[1.] IB, Instituto Butantan, Avenida Vital Brasil 1500;

Background: Snakes of the *Bothrops* genus are the main cause of snakebite accidents in Brazil. The pathogenesis of *Bothrops* envenomation is composed of local and systemic effects promoted by protease-rich venoms. The fibroblasts, important components of connective tissue, act in the healing of inflammatory and traumatic injuries, thus the identification of the alterations caused by the effects of toxins on these cells is essential to understand the molecular pathways related to venom-induced pathological changes. Thus, the aim of this project is to characterize the secretome of murine fibroblasts (BALB/3T3 clone A31 - ATCC cell line) after exposure to *B. jararaca* venom, by mass spectrometry-based proteomic analysis.

Methods: $4x10^6$ 3T3 cells were incubated with two concentrations of *B. jararaca* venom (0.5 and 1.0 ug/mL) for 2 h. To evaluate changes in protein abundance, proteins of the secretome were subjected to mass spectrometric analysis by LC-MS/MS. To this end, samples were submitted to reduction and alkylation and digested with trypsin using the SP3 protocol. LC-MS/MS was carried out using a Vanquish Neo UHPLC system coupled with an Exploris 480 mass spectrometer, under data independent acquisition. Analyses were carried out with DIANN beta 1.8.2 and statistical analysis were performed in R with scripts specific for this project.

Results: A total of 2,307 unique proteins were identified, and a total of 956 were quantified in all samples, and differential abundance of 36 and 209 proteins was detected in the secretome of cells incubated with, respectively, 0.5 and 1.0 ug/mL of venom. Proteins related to cell-cell adhesion, proteolysis, cell signaling and regulation of protein secretion, for example CREB3 regulatory factor and COP9 signalosome complex subunit 6, were observed with decreased abundance. Additionally, there was an increase in the abundance of proteins related to cellular response to fibroblast growth factor stimulus, apoptotic process, collagen-activated tyrosine kinase receptor signaling pathway and oxidative phosphorylation, for example latent-transforming growth factor beta-binding protein 2, cytochrome c oxidase subunit 6B1 and EMILIN-1.

Conclusion: These preliminary results reveal the early response of fibroblasts to *B. jararaca* venom toxins as viewed by alterations in proteins of different pathways, present in the secretome.

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