Bothrops jararaca snake venom effect on HeLa cervical cancer cell line

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Introduction. Cervical cancer is the third most common type of cancer in women, and the fourth to cause the highest number of deaths among them. HeLa cell line originally isolated from a patient with cervical cancer have special resistance and they are capable of multiplying indefinitely, being widely used in laboratory studies. Several works in the literature have reported that components of snake venom including *Bothrops jararaca* present potential tumor suppressors. In fact, previous work from our group showed that *B. jararaca* venom were able to modulate several proteins related to cancer cell metabolism, immune response, and inflammation in breast cancer cell lines.

Objectives. We aim to analyze and characterize the effect of *B. jararaca* venom on cervical HeLa cell line and characterize the changes in the protein profile by mass spectrometry in order to determine specific proteins or pathways affected by the venom.

Methods: HeLa cells were maintained at 37 °C in 5% CO_2 in DMEM culture medium supplemented with 10% inactivated FBS, ampicillin and streptomycin antibiotics. Upon reaching 80% confluence, cells were starved for 24 h before treating the cells with venom at low 0.63 mg/mL and high 2.5 mg/mL sub cytotoxic doses for 24 h. For the control, cells were treated with PBS. Cells were lysed with cold 8M urea supplemented with protease and phosphatase inhibitors. Samples were then reduced with DDT, alkylated with IAA, digested with trypsin and desalted using Stagetips. The obtained peptides were analyzed in Orbitrap Exploris 480 mass spectrometer coupled to a Vanquish Neo UHPLC.

Results: We were able to observe significant morphology changes after six hours of venom treatment, with some signs of loss of adherence. Furthermore, mass spectrometry-based proteomic analysis allowed us to identify 3079 different proteins in all samples analyzed from which 2017 proteins were identified in common to all different conditions tested. Comparing low and high dose of venom treatment against the control group revealed 170 proteins with fold change (FC) > 2 and 396 proteins with FC< 0.5 at 2.5 mg/mL of venom treatment and 254 proteins with FC <0.5 and 614 proteins with FC > 2.0 at 0.63 mg/mL of venom treatment. Among them, we highlight Txnrd1, Cdk, Mgst3, Rps6, Nras, Gnb2, Egf, Gstm3, Mapk1, Calm, Itga2, Grb2, Cycs, Tpm3, Ctbp2, Hsp90, Hdac2, and Rela which are all related to cancer.

Conclusion. Label free semi-quantitative proteomic analysis allowed us to identify several proteins that play important roles related to cancer such as cell proliferation, invasion, metastasis, apoptosis, and stress response. These data show that *B. jararaca* venom or some of its components may modulate proteins related to cancer. A deeper proteomic characterization and systemic analysis of the set/ family of identified proteins, may allow one to better understand how the venom acts on this cell type and draw a way to modulate or halt the growth and development of cancer.

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