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DOI: 10.51219./clinicalpharmaforum.2020.17.Martins-Ferreira

International E-Conference on

PHARMACOLOGY & TOXICOLOGY

December 01-02, 2020 | Webinar

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What does Bufalin tell us about Changing Shape?

Cardiotonic steroids (CS) are now known to bind to Na+/K + -ATPase located in caveolae and transduce the extracellular signal to intracellular compartments via activation of different protein kinases, including Src tyrosine kinase-Ras-ERK1/2 pathway. Consequently, they regulate cell cycle and gene expression, thus playing an important role in the control of renal and cardiac functions. Fibrosis is characteristic of the advanced stages of chronic renal and cardiac disease. Epithelial to Mesenchymal Transition (EMT) is a biological process involved in the establishment of fibrosis. One of the first signs of EMT is the endocitosis of membrane E-cadherin and impairment of cell-cell adhesion followed by gene activation of SNAIL which in turn inhibits E-cadherin and enhances mesenchymal proteins, as vimentin gene transcription, resulting in a phenotype transformation. Src kinase has been shown to contribute to EMT in cancer. Recent studies have identified both ouabain and marinobufagenin as endogenous steroids whose production and secretion are regulated by multiple physiological and pathological stimuli including angiotensin II and epinephrine in human. This study aims to characterize the mechanism involved in CS causing EMT. For this purpose, LLC- PK1 epithelial cell cultures were exposed to bufalin, a CS purified from toad venom, and EMT characteristics along with Src-Ras-ERK1/2 signaling pathway were evaluated. EMT characterization and signaling pathway were evaluated by phase contrast and fluorescent microscopy, high content screening microscopy, surface protein biotinylation and immunoblotting assays at 4 or 24 hours after incubation with 20 nM bufalin. Results: Bufalin 20 nM treatment for 4 or 24 hours increased endocytosis of E-cadherin as shown by immunofluorescence staining and a decrease of almost 45% of surface/total E-cadherin ratio in 20 nM bufalin- treated cells ($n=3$, $p<0.05$) evidenced by surface biotinylation. In 24hours we detected a change from epithelial to fibroblast-like morphology in bufalin-treated cells, and those cells expressed a considerably higher content of stress fibers, whose formation was prevented by inhibition of Src (2 μM PP2) and MAP kinases (10 μM U0126) activation as well as changes in cell morphology. ERK1/2 activation increases around 120% in cells treated for 24 hours. Discussion: The Na+/K + -ATPase bufadienolide ligand bufalin activates Src and MAP kinase signaling pathway and causes EMT in cultured renal cells. Loss of surface E- cadherin expression is in between the first steps of EMT phenomenon and is induced by bufalin. These results indicate that CS may be a key player in cell differentiation and fibrosis common in later stages of kidney chronic disease. Defining the intracellular pathways involved in

ISBN: 978-1-8382915-2-5

this process contribute to the development of novel pharmacological therapies.

Biography:

Jainne Martins Ferreira is the director of the science and health division of Neurogenesis Institute. As a postdoctoral student with François Noel at the Universidade Federal Rio de Janeiro she conducted a research about cardiotonic steroids and phenotype cell change. Previous she was a postdoctoral with Edward Ziff at the New York University conducting neuronal shape and studies. She joined Universidade Unigranrio School of Medicine, where she was Professor of Pharmaceutical Biotechnology and Neural Science and was an Investigator of the Translational Biomedicine Program.

ISBN: 978-1-8382915-2-5