Apoptosis Induction on *Rhipicephalus (Boophilus) microplus* Embryo Cell Line BME26

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The aim of this work is to describe the events related to apoptosis on the BME26 embryo cell line. This physiological process is one kind of cell death that is highly regulated by morphological and biochemical changes. Recent discoveries from our group point that the insulin signaling pathway (ISP) is present and plays important roles both on *Rhipicephalus (Boophilus) microplus* tick and in BME26 cells. Besides its metabolic role, ISP also transduces cell survival signals in several models. In order to observe the induction of apoptosis in the presence of ISP inhibitors tried to stablish apropriate controls by: I) growing factors deprivaton, II) generation of reactive oxygen species (ROS) and III) Ionizing radiation exposure. Preliminary results are based on morphological features observed on light microscopy by Giemsa staining, or fluorescence microscopy by Ethdium bromide (EB) alone or combined with Acridine Orange (EB/AO). When BME26 cells were kept up to 72 hours in medium without Fetal Calf Serum (FCS) they presented a reduced number of vesicles if compared with cells under normal conditions. Cell reduction was also observed in serum starved cells analyzed by flow-cytometry. Only a few apoptotic nuclei were observed in cells treated with H_2O_2 1 mM. Exposure to ultraviolet induced a generalized effect of small vesicles closely associated with the plasma membrane, suggesting blebbing formation. Nuclei condensation was also observed and was dose-dependent to U.V. exposure. Initial treatments with ISP inhibitors induced similar morphological alterations. Further studies on caspase activity and phosphatidyl-serine exposure are on the way to confirm apoptosis on BME26 cells.

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