**Study of the ARHGAP21 protein in autophagy induced by glucose deprivation in prostate cancer cells**

The ARHGAP21 is a RhoGAP protein with important functions in tumorigenesis, such as adhesion, migration and cellular proliferation. We observed that ARHGAP21 silencing alters the expression of genes involved in glycolytic pathway and autophagy. ~~Therefore~~ We evaluated the autophagy induced by glucose deprivation in prostate adenocarcinoma cells (PC3 and LNCaP) with inhibited ARHGAP21 expression. The cells were transfected with a specific interfering RNA (siRNA) for ARHGAP21 inhibition and cultured in medium with varying glucose concentrations (2000, 250, and 0mg/L) for 72 hours. The autophagy was evaluated using acridine orange by flow cytometry and the expression of autophagy-related genes was ~~were~~ studied by quantitative PCR and Western Blot. Apoptosis was also evaluated using anexin V by flow cytometry and mitochondrial activity was evaluated with a spectrophotometer.

We observed that glucose-deprivation stress induced autophagy, apoptosis and mitochondrial damage in both cell lines. Interestingly, LNCaP cells presented more autophagy and less apoptosis in the ARHGAP21-inhibited LNCaP cells when compared to the control sample, evidencing the anti-autophagic and pro-apoptotic role of the ARHGAP21 in this type of cell. Similar modulation in the autophagic process was not observed in PC3 cells.

In order to elucidate the ARHGAP21 relevance on the glycolytic pathway, its genes and protein expressions were also analyzed with real-time PCR and Western Blot. Furthermore, we also analyzed the expression of other proteins relevant to the autophagic process, namely, p62, BECLIN, and both LC3I and LC3II, owning to the importance of the LC3I conversion into LC3II during the formation of autophagosomal membranes. We observed that, in LNCaP cells, the glucose deprivation increased the expression of p62, BECLIN, and LC3, all of which are involved in the autophagic process. In LNCaP cells, we also observed an increase in the expression of the LC3II and p62 proteins when those cells had the ARHGAP21 protein inhibited. In PC3 cells, we observed that none of the autophaghy-related genes and proteins evaluated had their expression altered.