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Atomic force microscopy use in apoptosis investigation

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Due to the numerous advantages of atomic force microscopy (AFM), it is now applied to the investigation of surfaces morphology changes of variety of biological cells [1]. Apoptosis or programmed cell death is a process of controlled cell removal in multi-cellular organisms. Recent studies have demonstrated that reactive oxygen species (ROS) play a pivotal role in apoptosis. Although ROS have been implicated in cell death, the exact mechanism(s) are, as yet, unclear. Insulin-producing pancreatic β-cells have been known to be particularly sensitive to oxidative stress (as

pancreatic β-cells have been reported to be deficient in glutathione peroxidase, catalase, and superoxide dismutase relative to other tissues), making these cells useful models to study of oxidative stress-induced apoptosis mechanism(s).

For observations, the β-cells were cultured on polylyzin-coated glass slides. For AFM investigations, the β-cells with and without H_2O_2-treatment (0.5 mM solution) were fixed with 2% glutaraldehyde for 60 min and postfixed with 1% OsO_4 for 30min. The cells were then dehydrated in 30 – 100% ethanol and dried using a critical point dryer. Surface morphology of pancreatic β-cells with and without hydrogen peroxide treatment was investigated by a Nanoscope (R) IIIa MultiMode atomic force microscope. Images were captured in air using tapping-mode AFM.

The cell body without H_2O_2-treatment extends in range of ~ 10 μm and its height is about 2.5 μm. Zooming in on the cell the globular structure of the plasma membrane was examined. The *xy* size of the particles was generally between ~30 and 100 nm. Damages of the plasma membrane of the β-cells without H_2O_2-treatment were not observed. Cell shrinkage and cell fragmentation are visualised after the β-cell treatment with H_2O_2. Treated with H_2O_2 β-cell on glass flattens, its size in height is ~ 0.5 μm. On the cell surface the local membrane convexities are observed. Possibly, fragments of cell organelles are located under these convexities. Most likely, the formation of apoptotic bodies is visualised. The decreasing of scan area allows to observe plasma membrane blebbing originated from H_2O_2-treatment. The presence of typical features of apoptotic morphological changes of the β-cells treated with H_2O_2 as observed by AFM let us conclude that apoptotic cell death was observed. Previous studies have demonstrated that H_2O_2, most probably through the generation of ROS, causes an increase in intracellular calcium levels that precedes, if not causes, cell death in wide variety of cell types. There is no clear consensus in the literature indicating the likely mechanism by which H_2O_2 causes an increase in intracellular Ca^{2+} levels. Without doubt mitochondria, which are responsible for the initial calcium response under oxidative stress play major role in apoptosis. Further AFM investigations should be directed for the study of intracellular organelles surface morphology changes, in particular mitochondria under oxidative stress.

References

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