

## **INFLUENCE OF CELL ORIGIN IN-VIVO AND PROLONGED PROLIFERATION IN-VITRO ON THE ACCUMULATION OF THE MITOCHONDRIAL COMMON DELETION.**

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Mitochondrial DNA (mtDNA) mutations play a major role in human aging processes and degenerative diseases. The most frequently reported mutation of the mtDNA is the 4977-bp Common Deletion. There is strong support that Reactive Oxygen Species (ROS) generated by fractional univalent reduction of O<sub>2</sub> during respiration in mitochondria are responsible for the generation of mtDNA mutations. We quantified and compared the incidence of the Common Deletion in human chondrocytes, fibroblasts and keratinocytes. Furthermore we showed the effect of prolonged proliferation of chondrocytes, fibroblasts and keratinocytes on the accumulation of the Common Deletion. We established a quantitative real-time polymerase chain reaction assay using the TaqMan™ system to detect the Common Deletion. We optimised this assay by constructing plasmid external calibration standards for absolute quantification of the Common Deletion by standard curve method. Quantification of total mitochondrial DNA and the 4977-bp Common Deletion was performed to assay the percentage of the Common Deletion. We found that the accumulation of Common Deletion in chondrocytes, fibroblasts and keratinocytes is not age-related. We also showed that there is significant less Common Deletion in chondrocytes than in fibroblasts and keratinocytes. Furthermore we showed that prolonged proliferation of fibroblasts and keratinocytes led to a distinct reduction in the amount of Common Deletion whereas in chondrocytes it has little effect. These results are also of importance for autologous cell transplantation whereby prolonged proliferation in-vitro could lead to implantation of rejuvenated cells. This effect might be due to more slowly replication of damaged mitochondria because of impaired energy generation and reduced proton gradient and therefore the outgrow by intact mitochondria in mitotically active cells.