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## Evidences of existence of two different populations of hypertrophic chondrocytes in equine growth cartilage

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Osteochondrosis is a developmental orthopedic pathological condition results from a failure in the normal process of endochondral ossification and associated with retention of cartilage foci within the subchondral bone. A better understanding of endochondral ossification is important for understanding the pathogenesis of osteochondrosis. In the process of endochondral ossification, chondrocytes in growth cartilage undergoes proliferation, hypertrophy and death. Early ultrastructural studies described two morphologically different types of hypertrophic chondrocytes, dark and light. The subsequent publications have ignored the observations of diversity of hypertrophic chondrocytes, and assumed chondrocytes are homogenous cell population. Few recent studies described dark (but not light) chondrocytes. The current study was undertaken to investigate whether light and dark chondrocytes represent different stages of differentiation or two different populations of hypertrophic chondrocytes. Growth cartilage from foetal and postnatal foals was examined with light and electron microscopy. Chondrocytes were isolated from the growth cartilage of foetal foals and cultured as 3-dimesntional (3-D) pellets with 10% foetal calf serum (FCS) or triiodothyronine (T3) for 25 days. The pellets were examined by light and electron microscopy and quantitative real time polymerase chain reaction (Q-PCR). Electron microscopic studies of the equine growth cartilage revealed the existence of dark and light chondrocytes in all samples examined. The proportion of each cell type was similar at the late proliferative and late hypertrophic zones. In pellet culture, the two types of cells were observed. In 10% FCS, the majority of cells were dark, however, in T3 many light cells were found. Pellets with a higher proportion of dark chondrocytes expressed significantly higher levels of VEGF and MP-13 mRNA, but collagen type II and Runx2 mRNA expression was higher in pellets with a higher proportion of light chondrocytes. These observations suggest that hypertrophic dark and light chondrocytes are different cell populations not only morphologically but also at the molecular level. The culture system may be used for further studies on dark and light chondrocytes.