EVALUATION OF IMMORTALIZED HUMAN MESENCHYMAL STEM CELLS AT DIFFERENT POPULATION DOUBLINGS LEVELS

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INTRODUCTION: Immortalized cells are often used for in vitro studies, but proliferation and differentiation of the cells may vary considerably at different population doublings levels (PDL). The aim of this study was to characterize differences between early and late PDL. MATERIALS AND METHODS: Telomerase-immortalized hMSCs at PDL 180-189 (early) and PDL 274-283 (late) were cultured in 10%FCS-DMEM (control). Proliferation was determined using SYBR green assay. Cells stimulated by calcitriol were analysed for ALP activity on day 4 and 7. Control medium containing dexamethasone, ß-glycerophosphate and ascorbic acid was used to induce osteogenic differentiation, and calcification was assessed using alizarin red staining after 1, 2, and 3 weeks. RESULTS: The proliferation was stronger in late versus in early PDL (PD time 1,5 versus 2,3 days). ALP activity was lower in early PDL at all conditions and time points. Calcitriol supplemented medium induced higher ALP levels than control medium, the ratio calcitriol/control being highest for early PDL. Late PDL increased the ALP during culture independent of calcitriol addition, whereas early PDL only increased upon stimulus. Early PDL responded strongest to the osteogenic stimulus as verified by alizarin red. Results from studies of in vivo ectopic bone formation in mice are pending. CONCLUSION: Differences were observed between the various PDL. Early PDL had a lower proliferation, but higher capacity for osteogenic induction, as compared to late PDL. Therefore, careful consideration regarding PDL is needed when studying immortalized cells.