Monitoring Senescence in a Human Primary Fibroblast Cell Line Using Raman Micro-spectroscopy

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Senescence is a biological process of aging, which is associated with changes in molecular and cellular structures. Internal and external influences affect dominant specific protein interactions and cellular pathways. Thus, cells might participate in biologically complex processes with opposing effects. These are ageing, self-destruction (apoptosis), tissue repair and tumor suppression or promotion [1]. Both organ and cellular senescence can occur. In normal cultured diploid cells, the process of cellular senescence is marked by a finite number of proliferations [2]. Normally, cells stop proliferating after about 55-80 cell divisions (counted in population doublings; also known as 'cellular or replicative senescence' or 'Hayflick limit`). Remaining senescent cells are metabolically active displaying specific phenotypes. Typical morphological changes combined with cellular senescence include: increased cell size [3] associated with changes in cytoskeleton, or shortening of telomeres. Detection and identification of senescent cells *in vitro* and *in vivo* are based on a few biomarkers. Diagnostically important senescence markers in human fibroblasts include induction of senescence-associated β-galactosidase [4] or secretion of cytokines IL6 and IL8 [5]. Since none of these markers by itself can reproducibly report the senescent state, it is necessary to identify further - more reliable - identification methods. BJ cells (primary human fibroblast cells from the foreskin of a newborn male with a healthy status) were maintained in culture until they reached senescence. At certain time points, cells were fixed on Calcium fluoride cover slips. From these fixed cells, Raman micro-spectroscopic images were generated. The obtained maps were subjected to cluster analysis, and the information of the pseudo-color cluster images was used for further studies. Compared spectra from young/proliferating and senescent cells demonstrated that Raman spectroscopic imaging might be an efficient tool in identification of the senescent state in single cell analysis.

This research was provided by grants of the Carl-Zeiss-Stiftung.

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