Raman spectroscopy for the characterization of Glioblastoma cells

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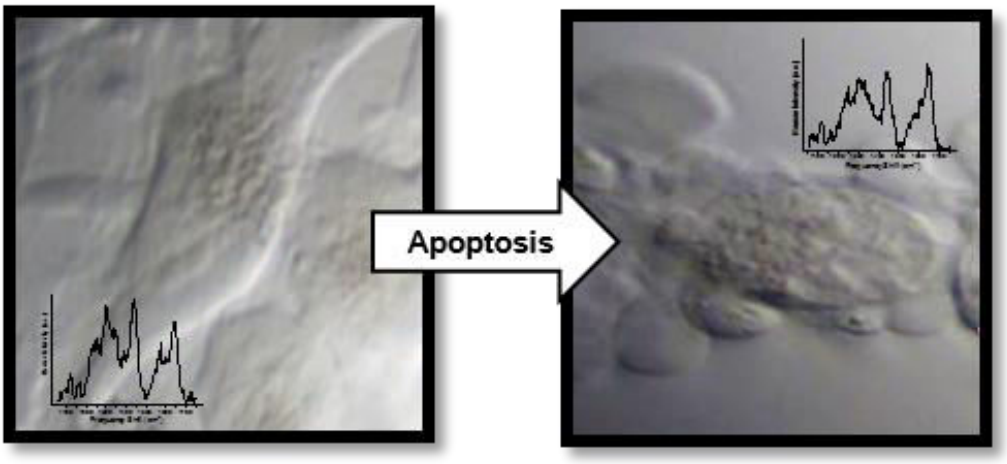
Micro-Raman spectroscopy has been recognized to be a powerful tool for bioanalytical and biomedical applications because it provides molecular information without external markers such as stains or radioactive labels [1]. In addition, Raman spectra allow the assessment of the overall molecular constitution of biological samples, based on specific signals from proteins, nucleic acids, lipids, carbohydrates [2]. Because most diseases initially start at the level of single cells, the ability to analyze individual cells in their physiological environment, without harming or modifying their state, has become ever more important [3]. Moreover, the possibility to probe individual apoptotic or altered cells is the principal advantage of the Raman spectroscopy, compared to the immunocytological and biochemical assays that depend upon large numbers of cells. In this study we analyzed living cells growing as adherent monolayers; in particular we selected the human glioblastoma cell line U251, a standard model for *in vitro* studies of neuronal growth and neurodegenerative diseases. Raman microspectroscopy was used to identify molecular markers for label-free monitoring of the glioblastoma cell viability in order to analyse the dynamic death events in single cells. We observed significant spectral variations correlated with visible morphological modifications, which are a consequence of molecular changes induced by nutrient depletion and the addition of an apoptotic inducer. In particular, the alteration in lipid metabolism during the apoptotic process is reflected in a shift of the lipid Raman features and this is closely related with the formation of apoptotic bodies. The measurements revealed also the translocation of the cytochrome c from mitochondria to the cytosol in nutrient-depleted cultures that is indicative of the cell suffering condition [4]. In order to monitor the subcellular distribution of the major cellular constituents during the death process, we obtained chemical images on glioblastoma cells in different states of degeneration. Raman maps show the progressive dispersion of the chromatin in the cytoplasm that can be associated with the dissolution of the nucleus and consequently with the cell death. This work confirms the potential of Raman spectroscopy that arises from its ability to detect biochemical changes at a molecular level and therefore, to be possibly used for diagnostics or as a tool for evaluating new therapies.



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