FTIR microspectroscopy of single proliferating eukaryotic cells

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Spectral differences between normal and abnormal tissue observed to date appear to be due to different averaging processes of spectral patterns that differ according to the cell's biochemistry, due to its state of maturation, differentiation, and development. In this scenario, a disease process would perturb the distribution of cells in the different stages of maturation, differentiation, and development. Previous FTIR microspectroscopic studies of normal *vs.* neoplastic cells and tissues have shown differences in the absorption intensities and band-shapes, particularly in the low frequency (900-1200 cm^{-1}) spectral region. To fully understand these differences, we must understand the spectral changes due to the drastic biochemical and morphological changes occurring as a consequence of cell proliferation.

The cell cycle is defined as the interval between the completion of mitosis of the parent cell, and the completion of mitosis in one or both of the daughter cells. It is divided into four main phases: G1, S, G2 and M, each representing a period with a particular function. Whole-culture synchronization methods have been used for many years in the field of cell biology to study events during the cell cycle and are accepted as valid approaches to cell-cycle analysis. We are studying the cell-cycle dependence of the FTIR and Raman spectra of single cells to determine the spectral patterns of individual cells as a function of its state of maturation, differentiation, and development. To accomplish this, we forego any chemical perturbations leading to cell-cycle arrest in efforts to synchronize cells, but employ a "mitotic shake-off" to select cells in mitosis and follow them through a time course of about 31 hours. This permits us to pinpoint the biochemical age of the cell and confirm its age by immunohistochemical staining (IHC) to detect specific cellular events, that include the appearance (and subsequent disappearance) of cyclins E and B1 and the uptake and incorporation of the thymidine analog 5-bromodeoxyuridine (BrdU) into nascent DNA during synthesis.

A positive (red) staining for cyclin E indicates that the cell contained sufficient amounts of cyclin E for detection at the time they were fixed. Cyclin E is a nuclear protein synthesized during the G1 phase, which reaches its maximum concentration at the end of G1 and is quickly degraded during the early S phase. A positive (green) staining for BrdU indicates that the cell was in S phase at the time of fixation, having incorporated BrdU into the genomic DNA. A positive (yellow) staining for cyclin B1 indicates that the cell contained sufficient amounts of cyclin B1 for detection at the time of fixation. Cyclin B1 is a cytoplasmic protein synthesized only in the G2 phase, reaches its maximum concentration at G2/M and is quickly degraded at the end of mitosis. Cells that exhibit a double-staining of red and green contain both incorporated BrdU and cyclin E, indicating that the cell was in the early stage of the S phase. Cells that exhibit a double-staining of yellow and green contain both incorporated BrdU and cyclin B1, indicating that the cell was in the early stage of the G2 phase.

Using IHC, we can confirm the biochemical age of the cell for comparing the FTIR spectral changes observed that are cell-cycle dependent.