Cellular Progression and Death: A Comparison of Spectroscopic and Cytofluorimetric Signatures

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Cellular proliferation, transformation and death are tiny regulated events at the basis of organism life and the interplay between them is of paramount importance for hindering diseases occurrence and progression and eventually being able to guarantee a successful therapy. For example, tumorigenesis is associated with several changes that alter cellular susceptibility to apoptosis while drug sensitivity is often dependent on the cell cycle stage. The knowledge of cell cycle phase distribution as well as of early apoptotic markers are of paramount importance for understanding cellular behaviour under normal and stressed conditions. These tasks are usually assessed using Flow Cytometry (FC) or immunohistochemistry which usually requires fixation. In this presentation, results and information obtained by FC and IRMS analysis of live cells for assessing the cell cycle stage distribution and induction of apoptosis will be compared.

In particular, asynchronous, S- and G0-synchronized B16 mouse melanoma cells were studied by running parallel experiments based on MD-IRMS and FC using Propidium Iodide (PI) staining of cellular DNA. Hierarchical Cluster Analysis (HCA) of cellular microspectra in the 1300–1000 cm^{-1} region pointed out a distribution of cells among clusters, which was in good agreement with FC results among G0/G1, S and G2/M phases. The differentiation was mostly driven by the intensity of PhI and PhII bands. In particular, PhI almost doubled from the G0/G1 to G2/M phase, in agreement with the trend followed by nucleic acids during cellular progression (1). The ability of IRMS to differentiate between G1-progressive and G0-resting phases will also be shown, a result proven by immunohistochemistry and achievable by FC only using a multi-probe approach.

The induction of apoptosis in U937 monocyte cell line by serum starvation and CCCP (carbonyl m-chloro phenylhydrazone) exposure was also followed by IRMS and FC. The percentage of viable, early and late apoptotic as well as necrotic cells was assessed first by biparametric PI-DiOC6 FC analysis and results were compared with the ones obtained by IRMS. Again, both techniques were concordant, but complementary: IRMS allowed getting details that FC could not easily reveal and vice versa. As an example, IRMS analysis allowed to establish that the apoptotic progression of U937 due to growth factor withdrawal is associated to the accumulation of lipid droplets, a mechanism revealed for other cell lines, leukemic and not, by NMR spectroscopy while the mitochondrial membrane depolarization detected by FC was not revealed by IRMS, that however had the ability to discriminate between reversible and irreversible mitochondrial damage.

Overall, both FC and IRMS are multiparametric techniques that permit the assessment of a broad range of information at the same time. Actually, IRMS is less selective and slower than FC, but it is a label-free technique that lately has become applicable on live cells thanks to the optimization of devices and methodologies. IRMS has been shown to be a suitable technique for achieving accurate information of cellular lipids, which usually degrade after the permeabilization techniques used for the promotion of dye penetration.

References:

[1] D. E. Bedolla, S. Kenig, E. Mitri, P. Ferraris, A. Marcello, G. Grenci, and L. Vaccari, *Analyst* 138, 4015–4021 (2013).