Testing drug resistance/sensitivity in leukemic cell lines by microFT-IR

Giuseppe Bellisola[1,2], Marzia Vezzalini[2], Claudio Sorio[2], Sara Redaelli[3], and Gianfelice Cinque[4].

[1] Azienda Ospedaliera Universitaria Integrata Verona; [2] University of Verona, Department of Pathology and Diagnostics, General Pathology Section, Strada Le Grazie, 8, I-37134 Verona, Italy; [3] University of Milano-Bicocca, Department of Clinical Medicine and Prevention, S.Gerardo Hospital, I-20052 Monza, Italy; [4] Diamond Light Source Ltd, Diamond House, Harwell Science and Innovation Campus, Didcot, Oxfordshire, OX11 0DE, United Kingdom

Background. Fourier Transform (FT) InfraRed (IR) microspectroscopy (microFT-IR) is an accurate, reliable and cost-saving analytical techniques with many potential applications in biomedicine. Nevertheless, it continues to be 'under strong development' or 'promising' and no microFT-IR system has been put in pre-clinical drug screening of new candidate drug molecules or in clinical trials. **Aims**. To demonstrate that microFT-IR in combination with unsupervised multiparametric analysis is a robust method to achieve rapid and accurate information about drug-sensitivity/resistance in cancer cells. **Methods**. We performed IR analysis in human chronic myeloid leukema (CML) K562 and MEG-01 cell lines expressing the BCR-ABL tyrosine kinase (TK) [1]. Moreover, we considered also the mouse bone marrow-derived pro-B cell line BaF3 that survives in the absence of interleukein-3 (IL-3) only when cells express constitutively active BCR-ABL TK or other oncogenes. BaF3 cells stably transfected with human p210 BCR-ABL or with the V299L and T315I mutants of p210 BCR-ABL were utilized as model cells that are sensitive or resistant to the TK inhibitors (TKIs) STI571 (imatinib-mesylate, a gift from Novartis Pharma, Basel) and BMS354825 (dasatinib), respectively [2]. In addition, also the drug compound FTY720 (also known as Fingolimod) acting on a different pathway [3] was also utilized in time-course and dose-response experiments with K562 and MEG-01 cells. Cells were fixed in 1% buffered formalin, washed with distilled water, and then deposited and dried in homogeneous manolayer on ZnSe window. The mid-IR absorbance spectra of several single cells were acquired at the Infrared Microspectroscopy end-station of B22 beamline, Diamond Light Source, using either Synchrotron Radiation and Globar as IR sources. Initially univariate analysis was performed on spectral components identified and assigned according the so-called "group frequency approach". **Results**. Combining IR spectral data with the results of complementary biochemical investigations carried out in samples we were able to identify and cross-validate IR signatures of drugs targeting the oncogenic protein BCR-ABL and its associated abnormal tyrosine kinase activity in CML blast cells [4]. Unsupervised pattern recognition performed by Hierarchical Cluster Analysis (HCA) applied to the spectra of untreated leukemic cells identified two distinct final groups roughly corresponding to living and to apoptotic cells, respectively. The corresponding IR spectral profiles were assumed to represent drug-resistant and drug-sensitive cells. Using these IR markers we verified that microFT-IR in combination with HCA was able to segregate in drug-sensitive from drug-resistant cells. **Conclusions**. MicroFT-IR can be utilized in pre-clinical drug screening/testing as well as it may represent an useful tool in the monitoring of therapy follow-up in leukemic patients.

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