Testing Drugs Targeting Basic Defect in Cystic Fibrosis (CF) Epithelial Cell Lines by FTIR Analysis

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Background The basic defect in Cystic Fibrosis (CF), a multi-organ hereditary disease, relies on the impaired expression/activity of the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) protein on the plasma membrane of secreting epithelia. It is caused by thousands different mutations in the human CFTR gene and the most frequent is the F508 deletion (F508del) responsible for the reduced expression and function of CFTR. New molecular compounds promoting the trafficking of defective F508del/del CFTR to the proper place in the airway cell membrane (VRT-325, VRT-809) or improving its function as a chloride channel (VRT-770) need to be tested in *ex vivo* cell systems before to propose their evaluation in clinical trials. Unfortunately, current methods of assaying CFTR either cannot be applied ex vivo (sweat test) on CF cell models or are time-consuming needing extended manipulation of the sample (patch-clamp and single-cell fluorescence imaging via DiSBAC2(3)). Fourier transform (FT) Infrared (IR) microspectroscopy (microFTIR) should be a valid alternative to obtain quantitative and qualitative information on whole molecular composition and biochemical changes in cells without the use of specific fluorescent probes and/or other forms of sample pre-treatment. In association with methods for unsupervised multivariate data analysis, microFTIR has proved to be an accurate method to classify cells and to identify drug-induced biochemical changes in cell cultures [1-3].
Aims. To obtain proof of principle that microFTIR in combination with unsupervised multivariate data analysis is suitable for testing the effects of molecular compounds targeting defective CFTR.
Methods. The FTIR absorbance spectra of several individual formalin-fixed F508del/del CFTR-defective CFBE41o- and normal control 16HBe14o- epithelial cells uniformly spread on a ZnSe window were acquired in transmission mode at the DLS B22 IR beamline MIRIAM. Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) multivariate data analysis techniques were applied to explore dataset to identify relevant variables and classify groups of cells. To validate results interpretation, IR data were compared with those of complementary investigations carried out in replicate samples. In particular CFTR expression was detected by flow cytometry [4] and its functioning as chloride transported was measured by single-cell fluorescence imaging with the trans-membrane potential-sensitive DiSBAC2(3) probe [5].
Results. CFBE41o- and 16HBe14o- cells not exposed to correctors were classified separately as well as the spectra of untreated controls CFBE41o- and the spectra of CFBE41o- cells exposed to correctors VX-325, VX-809 and of ABT-888, a poly ADP ribose polymerase (PARP) inhibitor.
Conclusions. Initially developed for targeting cancer cells, ABT-888 has proved to be active also in the rescuing of defective CFTR as well as it seems less toxic than other correctors as indicated by IR data validated by the results of complementary investigations.

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References

[1] G. Bellisola et al., *Analyst* 140, 4407-22 (2015).
[2] G. Bellisola et al., *Analyst* 138, 3934-45 (2013).
[3] G. Bellisola et al., *Analyst* 135, 3077-86 (2010).
[4] J. Johansson et al., *Cytometry A* 85, 611-20 (2014).
[5] C. Sorio et al., *PloseOne* 6:e22212 (2011).