Investigation of Polyphenols Anti-cancerous Action According to their Differential Effects on Breast Cancer Cell Lines by FTIR Spectroscopy

A. Mignolet[1], F. Buytaers[1], V. Mathieu[2], E. Goormaghtigh[1]

[1]Laboratory for the Structure and Function of Biological Membranes, Center for Structural Biology and Bioinformatics, Université Libre de Bruxelles (ULB), Belgium
[2]Laboratoire de Toxicologie, Institut de Pharmacie, Université Libre de Bruxelles (ULB), Brussels, Belgium

Polyphenolic compounds constitute one of the largest groups of plant metabolites. It displays a vast array of molecular structures and cellular effects. In the past few decades, significant advances enabled researchers to investigate the potential use of phytochemicals to treat or manage a plethora of chronic diseases including inflammatory diseases, cardiovascular abnormalities and various types of cancer.[1] In fact, antitumor properties of some polyphenols such as curcumin have been already demonstrated as they can affect the different stages of carcinogenesis. Traditionally in pharmacology, new anticancer drugs are evaluated for their potential to inhibit the proliferation of cancer cell lines (IC_{50} measurement). This approach is obviously not sufficient, and molecules with new modes of action are especially needed. Considering the number of natural polyphenolic compounds already discovered and their numerous actions against cancer, it could be interesting to compare their differential effect on cancer cells. In the end, a preliminary classification of these molecules, based on the drug-induced cell metabolic modifications, could be established. Such a classification would help to combine molecules acting on diverse signal transduction pathways and foster synergies. The aim of this work is then to investigate and compare the differential effects of various polyphenols on cancer cells by infrared spectroscopy and to use it as a prediction tool for the effects of unknown polyphenols. To achieve this, infrared spectra of cultivated cells exposed to polyphenols at their IC_{50} are suggested to provide a precise signature of the "mode of action". It has been recently demonstrated that the infrared spectrum of cells exposed to anticancer drugs can offer a representative fingerprint of the metabolic changes induced by the drugs.[2–5] Interestingly this new systemic approach characterizes all the information from the genome, the proteome, the lipidome and the metabolome.

We examined the effects of various polyphenols on the epithelial breast cancer cell line MDA-MB-231, derived from a metastatic site. In a first step, the IC_{50} at 72 hrs incubation time is determined for each polyphenol using the cell viability ATP assay. For FTIR spectroscopy, the cancer cells are then exposed to the IC_{50} concentration of each polyphenol for 24 hrs. Cells are then harvested, washed in an isotonic solution and deposited on ZnSe 96 wells plate. Finally, infrared spectra are recorded using an HTS-XT connected to a Bruker FTIR spectrometer. The microplate extension HTS-XT allows high-throughput screening by FTIR spectroscopy. Spectra are processed with the program "Kinetics" running under Matlab. Unsupervised multivariate statistical analyses such as principal component analyses and supervised statistical analyses such as partial least square discriminant analyses are used to compare the different polyphenols.

References

[1] A.Y. Issa, S.R. Volate, and M.J. Wargovich, *J. Food Compos. Anal.* 19, 405–419 (2006).
[2] A. Derenne, R. Gasper, and E. Goormaghtigh, *Analyst* 136, 1134–41 (2011).
[3] A. Derenne, M. Verdonck, and E. Goormaghtigh, *Analyst* 137, 3255–64 (2012).
[4] R. Gasper and E. Goormaghtigh, *Classification of drug modes of action on cancer cells by FTIR fingerprinting*, IOS Press (2012).
[5] A. Derenne, V. Van Hemelryck, D. Lamoral-Theys, R. Kiss, and E. Goormaghtigh, *Biochim. Biophys. Acta* 1832, 46–56 (2012).