Analysis of human cancer cells and their response to antitumour drug treatment by Raman and infrared microspectroscopies

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Every year about 10 million people are diagnosed of cancer and more than 6 million die of it, which represent over 22 million of persons with cancer disease in the world. Currently, cancer is diagnosed via biochemical assays which look for abnormal levels of tumour markers; and biopsy examination, which is almost always necessary for confirmation. Although used routinely, the analysis of tissue and cytology samples to diagnose cancer is a laborious, time consuming, costly, and sometimes subjective methodology. Due to these facts, there is a real need for methods that are more reliable and less prone to erroneous diagnostics. It is known that early diagnostics and effective monitoring can influence patient care and the outcome. Over the last decade, vibrational spectroscopy has developed into an important biomolecular probe to study cells and tissues. It appears as a promising alternative to improve therapies and diagnostic methods in medicine. Furthermore, the possibility of studying cancer cells at sub-cellular level makes it an appropriate tool to characterize spectral biomarkers for cancer diagnosis. This could translate into the application of this methodology in clinical practice for diagnostic purposes. Assessment of cell response to anticancer agents is a very important issue in the management of patient treatment. Indeed, access to early cellular events in response to drug treatment, and in particular at low doses, would be of utmost importance in view of reducing chemotherapeutic side effects. In this work we have used vibrational spectroscopy to monitor the effects of weak, non-cytotoxic doses of an anti-cancer drug on a cancer cells. We used as model the non-small lung cancer cell line, Calu-1, exposed to cytostatic doses (0.1 to 100nM for 24h, 48h and 72h) of gemcitabine (Gemzar®), an antitumor drug, analogue of deoxycytidine, currently used in treatment of lung cancer patients. In these conditions, we found that inhibition of cell proliferation ranges from weak (≤5%), moderate (~23%), to high (82-95%) without affecting cell viability. After drug treatment at these doses and incubation times, spectra were recorded on cell populations as well as on single cells using respectively a conventional IR source and a synchrotron source. Our results show that spectral cell response to gemcitabine is detectable at sublethal doses and that effects observed on single cells are comparable to those of cell populations. Using cluster analysis, spectra from both sets could be classified in two main groups: a first group that contains spectra of cells exhibiting a weak or moderate proliferation rate, and a second group with spectra from cells presenting a high growth inhibition. These results are promising since they show that effects of subtoxic doses can also be monitored at the single cell level with the implications that this may have at a clinical point of view in terms of patient benefit and response to chemotherapy. We also explored the possibility of using Raman microspectroscopy for investigating single live cells. Differences in the biochemical and molecular composition between sub-cellular regions were assessed. In a perspective of future oncology, the potentials of vibrational spectroscopy in the field of metronomic chemotherapy and new modality of drug administration will be discussed.

References:

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