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Infrared spectra of primary melanomas can predict response to chemotherapy: The example of dacarbazine

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ABSTRACT

Metastatic melanomas are highly aggressive and median survival is 6–9 months for stage IV patients in the absence of treatment with anti-tumor activity. Dacarbazine is an alkylating agent that has been widely used in the treatment of metastatic melanomas and that could be still used in combination with targeted or immune therapies. Indeed, therapeutic benefits of these treatments in monotherapy are poor and one option to improve them is to combine drugs and/or to better anticipate the individual response to a defined treatment. To our best knowledge and to date, there is no test available to predict the response of a patient to dacarbazine. We show here that examination of melanoma histological sections by infrared micro-spectroscopy reveals the sensitivity of the cancer to dacarbazine. Unsupervised analysis of the FTIR spectra evidences spontaneous and significant clustering of infrared spectra into two groups that match the clinical responsiveness of the patients to dacarbazine used as a first-line treatment. A supervised model resulted in 83% of the patient status (responder/non-responder) being correctly identified. The spectra revealed a key modification in the nature and quantity of lipids in the cells of both groups.

1. Introduction

Melanoma is the most lethal form of skin cancer and its incidence is rising faster than that of any other cancer [1,2]. While early stages of melanoma have an excellent prognosis following curative surgery, metastatic melanomas are highly aggressive and median survival is 6–9 months for these patients [3–5]. Therapeutic agents targeting the MAP-kinases pathway or immune therapies (anti-CTLA4, anti-PD-1 drugs) have been shown to significantly increase survival [6,7]. However, on the one hand, targeted therapies are associated with development of resistances, rapid relapses, and secondary progression, and on the other hand, response to immune therapies is limited to a subset of patients [8]. Unmet needs therefore remain for the treatment of patients with metastatic melanoma. One option is to combine old and new drugs to increase the therapeutic response [9] and/or to better anticipate the individual response to a drug or another [8].

Dacarbazine was approved by the FDA in 1975 for the treatment of metastatic melanoma as a single agent [4,8]. It is an alkylating agent and a member of the family of imidazole carboxamide derivatives with structural similarities to some purines [10,11]. The drug causes methylation of the DNA bases and these DNA lesions lead to cell death, mainly *via* apoptosis [10,12].

Overall response rate of dacarbazine calculated from 8 randomized trials from 1992 to 2006 reaches only 13.4% [13], and to our best knowledge and to date, there is no test available to predict the response of a patient to dacarbazine chemotherapy. Fourier transform infrared spectroscopy appears to be one of the most relevant tools to characterize *ex vivo* tissues because this technique provides complete information about the biochemical content of any biological sample by contrast with immunohistochemistry or other molecular techniques that target one macromolecule type at a time. Infrared spectroscopy is based on the interaction between infrared radiations and the covalent bonds of biological molecules. Within the mid-infrared range (4000–400 cm^{-1} or 2.5–25 μm), each organic function leads to specific absorption bands and each compound has a unique characteristic set of absorption bands (e.g. proteins, nucleic acids, lipids). Taken together, the various contributions to the FTIR spectrum form a signature of the molecular composition of the cell that is unique. When coupled with microscopy, Fourier transform infrared (FTIR) spectroscopy becomes a tool for histopathological studies [14–17] and provides spatially resolved information on the chemical composition of the sample [18]. Because of its

Abbreviations: FPA, Focal plane array; FFPE, Formalin-fixation paraffin-embedding; FTIR, Fourier transform infrared; IR, Infrared; MCT, Mercury cadmium telluride; MTIC, 5-(3-methyl-1triazeno)imidazole-4-carboxamide; PCA, Principal component analysis; PC, Principal component; PLS-DA, Partial least square discriminant analysis; S/N, Signal to noise.

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potential to probe chemical constituents without dyes or specific reagents, FTIR imaging is becoming a powerful tool to complement the existing diagnostic methods [15]. In cancer research, FTIR imaging has proven its value in the study of a large panel of different cancers and particularly in skin cancers [19–21]. To sum up, FTIR spectroscopy provides a powerful means of classifying tumors based on their underlying biology.

This study based on primary melanoma tissue sections from 5 responders and 7 non-responders to dacarbazine demonstrates that FTIR spectroscopy could be used to predict response to dacarbazine treatment.

2. Materials and methods

2.1. Patient samples

Primary melanomas from 12 metastatic melanoma patients were provided by the University Hospital of Angers (Angers, France). These primary melanomas were formalin-fixed and paraffin-embedded (FFPE). All these 12 patients received dacarbazine as chemotherapy after resection of the primary tumor. Patients were classified among two groups according to their response to dacarbazine assessed by PET-CT or CT scans 3 months after chemotherapy and then every 3 months. Five and 7 patients were classified as responders and non-responders, respectively. From each FFPE primary tumor, 2 sections of 4 μm were cut with a microtome. The first section was deposited on a BaF_2 slide (transparent to IR light, ACM, Villiers St. Frederic, France). This section was deparaffinized as described [22], and FTIR measurements were recorded. The second section, adjacent to the first one, was deposited on a glass slide and was stained by hematoxylin and eosin (H&E). This slide was used to localize the tumor areas by a pathologist.

2.2. FTIR measurements

The FTIR data were collected using a Hyperion 3000 FTIR imaging system (Bruker Optics, Ettlingen, Germany), equipped with a liquid nitrogen cooled 64 × 64 mercury cadmium telluride (MCT) focal plane array (FPA) detector, in transmission mode. The size of an image covers an area of 180 × 180 μm^2 and is composed of 4096 pixels of 2.8 × 2.8 μm^2 each. It must be noted that spatial resolution can be significantly lower than the pixel size, depending on the wavelength. It took about 5 min to record an infrared image composed of 4096 spectra at a spectral resolution of 8 cm^{-1} and where each spectrum is the average of 256 scans. As tumors and melanomas in particular are rather heterogeneous [23,24] and to decrease the likelihood of a measurement on a singular spot, we recorded 5 measurements (of 4096 spectrum) in 5 different areas of each primary tumor. This enabled us to take into account at best the heterogeneity of the tumors.

3. Data analysis

3.1. Preprocessing

All spectra were preprocessed as follows. Water vapor contribution was subtracted as described previously [41,42] with 1956–1935 cm^{-1} as reference peak and CO_2 peak was flattened between 2450 and 2250 cm^{-1}. The spectra were baseline corrected. Baseline correction improves the comparison between spectra. Indeed, slight shifts in the baseline can be observed, even among spectra from a same sample. Changing the sample section adds further (minor) changes to the baseline. It is therefore preferable to subtract a baseline to get rid of these effects that are not related to the tissue itself. Straight lines were interpolated between the spectra points at 3620, 2995, 2800, 2395, 2247, 1765, 1724, 1480, 1355, 1144, and 950 cm^{-1} and subtracted from each spectrum. Spectra were normalized for equal area between 1725 and 1481 cm^{-1} (amide I and II bands). The signal-to-noise ratio (S/N) was then systematically checked on every spectrum. It was required to be greater than 350 when noise was defined as the standard deviation in the 2000–1900 cm^{-1} region of the spectrum and the signal was the maximum of the curve between 1750 and 1480 cm^{-1} after subtracting a baseline passing through these two points.

Correction of the spectra for water vapor contribution, baseline subtraction and normalization, PCA, difference spectrum, and PLS-DA were carried out by Kinetics, a custom-made program, running under Matlab (Matlab, Mathworks Inc).

3.2. Difference spectra

Difference spectra allow emphasizing the spectral variations between two distinct conditions. Difference spectrum was built by subtraction of the spectra of melanoma cells of non-responders from spectra of melanoma cells of responders. A Student's *t*-test was used to reveal wavenumbers appearing significantly different among these two groups and are shown by black stars ($\alpha = 1\%$) in Fig. 3. Normality of the distribution of the absorbances was checked for each group at every wavenumber by a Kolmogorov–Smirnov test by comparison with a standard normal distribution, with a confidence level $\alpha = 0.5\%$ (not shown). The results demonstrated the normality of absorbance distributions.

3.3. Statistical analyses

Principal component analysis (PCA) was applied on the series of mean spectra of melanoma cells. As described previously [25], PCA is an unsupervised multivariate method enabling variable reduction by building linear combinations of wavenumbers varying together, called principal component (PC). The first principal component explains most of the data variance. The second principal component, uncorrelated to the first one, accounts for most of the residual variance and so on. Usually 2–6 PCs are sufficient to explain the major proportion of the original variance of the data set and reduce the description of each spectrum to 2–6 numbers representing the projection (scores) of the spectra on the PCs. One advantage of the method is that it extracts the important information as a set of new orthogonal variables, the principal components [26,27].

The multivariate analysis of the variance (MANOVA) indicates whether two groups are significantly different. The MANOVA was performed in this study on the first 6 PCs obtained after a PCA on the series of mean spectra from responders and non-responders.

Partial least square discriminant analysis (PLS-DA), a supervised analysis, was also conducted on the data set. PLS-DA was used to extract latent variables of the data set that enable the construction of a factor capable of predicting a class.

3.4. ROC curve

The receiver operating characteristic (ROC) curve is an effective method to assess the performance of a diagnostic test and represents the true positive rate (sensitivity) according to the false positive rate (1-specificity) across a series of cut-off points. These cut-off points are named threshold in this study and represent the percentages of spectra predicted as belonging to one class and for which we have to consider the patient in this class. This curve can be used in case of dichotomous test with two outcome categories. In our case, the two outcome categories are responders and non-responders. Sensitivity was calculated as $Sv = TP/(TP + FN)$ and specificity as $Sp = TN/TN + FP$, where TP, FN, TN, and FP correspond to true positive, false negative, true negative, and false positive, respectively [28].