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Discriminating nevus and melanoma on paraffin-embedded skin biopsies using FTIR microspectroscopy

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Abstract

FTIR microspectroscopy, in combination with cluster analysis, has been used to characterise skin tissues, in order to discriminate cancerous from non-cancerous ones. The main objective of this in vitro study was to demonstrate the applicability of infrared spectral imaging to separate, on paraffinised biopsies, pigmented nevi (benign skin lesions) from melanomas (malignant skin lesions). Infrared spectra were collected from paraffin-embedded samples of nevi and melanomas, without deparaffinisation. Despite the important contribution of the paraffin in these spectra, it was possible to find meaningful and discriminating spectral regions. Spectral imaging was first performed to localize different skin layers (dermis and epidermis). Spectra extracted from the images were subjected to hierarchical classification algorithm, which allowed the discrimination of melanomas from the nevi, using selected spectral windows that correspond to vibrations of DNA and melanin content. The diversity of skin lesions and direct accessibility to the skin make this organ an interesting field of investigation using this technique.

Keywords: Paraffinised tissue; Skin lesion; Melanoma; Nevus; Infrared; Microspectroscopy; Cluster analysis

1. Introduction

The skin is a stratified organ composed of three layers: epidermis, dermis, and hypodermis. The outermost layer is the epidermis, with an average thickness of 120 μm, but it could be much thicker on the palm and the sole. The epidermis is constituted of cells arranged in several layers, the keratinocytes, which produce keratin and are responsible for the barrier function. Skin pigmentation derives from melanocytes which produce melanin. Both proliferating keratinocytes and melanocytes are located within the basal cell layer of the epidermis, overlying the superficial dermis; the main molecular constituents of the epidermis are keratin and melanin. Under the epidermis, the dermis consists of a 1200-μm-thick structure, mainly composed of collagen fibres (types I and III) and elastin fibres [1]. The deepest part of the skin, the subcutaneous layer (hypodermis), consists of loose connective tissue and adipocytes.

Including malignant melanoma, basal cell carcinoma, and squamous cell carcinoma, skin cancer is the cancer with the highest incidence worldwide [2]. Cutaneous melanoma is the most severe skin cancer and accounts for three-quarters of all skin cancer deaths [3,4]. Malignant melanoma is a cancer whose incidence and mortality rates are rising in many parts of the world where light-skinned populations live [4].

Whereas carcinomas derive from keratinocytes, melanomas, as benign pigmented nevi, originate from melanocytes. The development of most melanomas includes an intra-epidermal and superficial dermis phase (lateral growth), followed by a second step with vertical growth into the dermis (invasive phase) [5,6]. The prognosis of melanoma is related to early detection, which is difficult in numerous

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cases, particularly due to the difficulty to separate it from atypical nevi. Therefore, new and efficient non-invasive tools for the early diagnosis of melanomas remain of crucial interest in clinical practice. Several studies have reported the potential of vibrational spectroscopies, infrared absorption, and Raman scattering to characterise biological tissues. Moreover, in the last few years, many investigations were carried out to differentiate cancerous from benign tissues on sections of colon [7,8], cervix [9,10], stomach [11,12], breast [13], skin [10], and oral carcinoma [14], and also, in vivo by dedicated infrared or Raman probe, for the detection of colorectal cancer [15] and Barrett's epithelium in rat's oesophagus [16]. Generally, multivariate statistical treatment is applied to spectral data in order to extract relevant information, criteria that can be considered as functional spectroscopic descriptors of a particular pathology. In skin tumours, Raman spectra have been treated by neural network to discriminate four different types of skin lesions [17,18]. Pseudo-colour cluster images can be reconstructed to map precisely melanotic zone [19] or basal cell carcinoma [20]. The clusters are determined by principal components or artificial neural network analysis of the spectra, associated with a classification method. In addition to cancer characterisation, vibrational microspectroscopic investigations were performed on the skin to determine molecular concentration profiles and to map the distribution of exogenous [21] and endogenous molecules [22,23].

In view of developing a spectroscopic tool dedicated to the early diagnosis of melanomas, we carry out here a retrospective study to assess the feasibility of nevus/melanoma discrimination by FTIR imaging in combination with hierarchical cluster analysis.

Generally, in case of formalin-fixed, paraffin-embedded tissues, spectroscopists eliminate chemically the paraffin before any measurements because paraffin presents intense vibration bands, in both infrared and Raman signals. Despite the paraffin signal, our choice was to employ paraffinised tissue sections without deparaffinisation and rehydration and to use regions of the spectra where the paraffin signal is absent, so as to avoid possible chemical alterations of the biological constituents.

2. Materials and methods

2.1. Tissue sample preparation

Ten-micron-thick tissue sections were cut from paraffin-embedded biopsies. For this first investigation, which has been led as a blind study, six biopsies were supplied by the Dermatology Department of Reims University Hospital. The true nature of the samples was histopathologically revealed only after infrared analysis; they presented three different types of melanomas: lentiginous malignant melanoma (strictly intra-epidermal), superficial spreading melanoma (Breslow 0.75 mm), and acrolentiginous melanoma (Breslow 4 mm). The Breslow's index corresponds to the maximal thickness of the melanotic zone in the dermis. The three other samples presented two types of nevi, composed and junctional nevi.

Sectioning was done in such a way that all sections presented the epidermis totally affected by proliferating melanocytes. Thus, no normal zones were present inside the epidermis of all studied samples as histolgically controlled. Sections were fixed on ZnSe slides, suitable for infrared transmission analysis, either with a droplet of albumin (egg albumin diluted in water), usually employed in histopathological examination, or with a droplet of distilled water. Both fixation solutions were used in order to verify their possible effect on the spectral data.

2.2. Infrared instrument

The samples were analysed by a Perkin Elmer Spectrum Spotlight 300 FTIR Imaging System, using the "image" mode of the instrument. For each tissue section, an area of 2×2 mm^2 was defined to cover all skin structures, and an IR image was produced using a liquid nitrogen cooled, 16-pixel mercury cadmium telluride (MCT-A) line detector at a 25 μm/pixel resolution. The possibility of acquiring an IR image at a higher spatial resolution (6.25 μm/pixel) is offered when a more detailed structure is required on a particular smaller area. An absorbance spectrum was recorded for each pixel in the transmission mode. Before capturing the IR image, the ZnSe window is measured as a reference, and a background spectrum was collected for each of the 16 pixels. All spectra were recorded in the mid infrared region (4000–720 cm^{-1}) at 32 scans/pixel and a spectral resolution of 4 cm^{-1}; these conditions allowed to obtain good quality spectral data with acceptable recording time (4 h to map a 2×2 mm^2 surface). A visible image of the sample was also collected and the sampled zone was chosen from it.

The spectra of the reference products, such as melanin or type I collagen, were recorded using the "point" mode of the apparatus (this mode uses a second MCT detector placed in the same Dewar), with an aperture of 100×100 μm^2 (covering the whole detector surface) and with similar acquisition parameters with that of the "image" mode.

2.3. Statistical analysis of the FTIR data

Hierarchical cluster analysis (HCA) was performed with the OPUS software (Bruker Optik GmbH, Germany) to classify the spectral data. HCA aims at clustering the data according their degree of similarity (resemblance). The method consists of calculating the Euclidean distance between all the set of data.

The merging process (result) can be visualized in a tree-like diagram, which is called a dendrogram, presenting the regrouping of the spectra in clusters according to a heterogeneity scale. Inter-spectral distances are first calculated with first derivatives of amide I normalised spectra,