Evaluation of squamous cell skin carcinoma using ATR-FTIR spectroscopy associated to cluster analysis

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Abstract: Cluster Analysis were used as an unsupervised classification technique to differentiate FTIR spectra of normal and tumor skin. The results shown satisfactory separation in samples analyzed, highlighting the potential of the technique for diagnostic purposes.

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1. Introduction

Cancer is one of the diseases with highest mortality rates in the world, and the large number of cases predicted for the following years have been worrying the scientific community. Prevention and early diagnosis have fundamental importance to reduce these numbers; however detecting the disease at an early stage is a difficult task, complicating the treatment of the patient.

Currently used diagnostic techniques are based on the morphological characteristics presented by the biological tissue. In histopathology, which is the gold standard for cancer diagnosis, a physician establishes the diagnosis based on the criteria of ABCDE rules (asymmetry, border, color, diameter and evolution) [1]. Beyond the subjectivity of this technique, the physiological evidences of cancer present themselves only when the disease is already at an advanced stage. Thus histopathology is not fully effective: an auxiliary detection method of cancer is necessary to accurately determine the presence of cancer cells still in early stages.

In recent years, the Fourier Transform Infrared Spectroscopy (FTIR) has successfully characterized the biological tissues. Due to the information at the molecular level, the FTIR technique has great potential to differentiate neoplastic from normal cells, making it a possible and powerful diagnostic tool [2-4].

2. Objectives

This work evaluates the potential of Hierarchical Cluster Analysis in differentiate FT-IR spectra of normal skin from squamous cell carcinoma tissue.

3. Material and Methods

Sample Preparation

Swiss mice were submitted to chemical carcinogenesis with a single dose of DMBA (7,12-Dimethylbenz[a]anthracene, Sigma Aldrich, CAS number 57-97-6) and three weekly doses of TPA (12-O-Tetradecanoylphorbol 13-acetate, Sigma Aldrich, CAS number 16561-29-8) for 28 weeks [5]. SCC (squamous cell carcinoma) and normal tissue were fixed in formalin, dehydrated with xylene, washed in ethanol baths with increasing concentrations and mounted in wax blocks. Using a microtome, slices with 5 μm of thickness were obtained from FFPP (Formalin-fixed paraffin-processed) sections and placed in MirrIR low-E-coated glass for the spectroscopy analysis. Due to the spectroscopic contributions of paraffin in the range used, FFPP sections were submitted to de-waxed protocol. For this, FFPP sections were immersed in a series of baths consisting of two baths of xylene for 10 minutes and, one bath of ethanol absolute for 5 minutes. After this, samples were kept in a dissector for 24 hours.

FT-IR Spectroscopy

Infrared spectra data were recorded in the range from 4000 to 400cm^{-1} with a Thermo Nicolet 6700 Fourier transform infrared spectrometer, equipped with deuterated triglycine sulphate (DTGS) detector, Globar (MIR) source and KBr Germanium beamsplitter. The samples were placed in an attenuated total reflection (ATR) accessory and the spectra were obtained against air as a background at room temperature.The ATR crystal was carefully cleaned with ethanol after each measurement. Twenty scans were taken from normal and SCC tissue at a resolution of 4 cm^{-1} and averaged.

Statistical Analysis

Data were analyzed using Minitab 17software. Hierarchical Cluster analysis (HCA) were used as an unsupervised classification technique in order to explore and differentiate the data structure. The similarity of different clusters was defined by correlation coefficient distance and calculated by Ward's method. The results were presented in a dendrogram structure, showing the different groups obtained.

3. Results and Discussion

Figure 1 shows the average of the FT-IR spectra obtained for normal and tumor tissue.

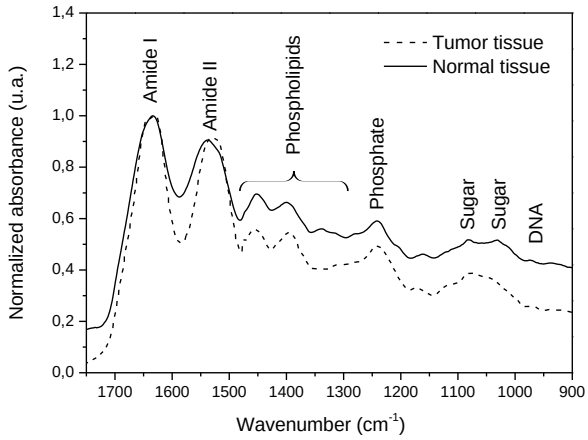

Figure 1. FT-IR spectra for normal skin and SCC tissue

In both spectra it is possible to identify the amide I and II bands. A shift in the amide II is observed and indicates changes in protein conformation. Tumor tissue displays an overall reduction in intensity for the bands associated to phospholipids, phosphate, sugar and DNA vibrational modes, which are associated with metabolic differences present by each tissue sample.

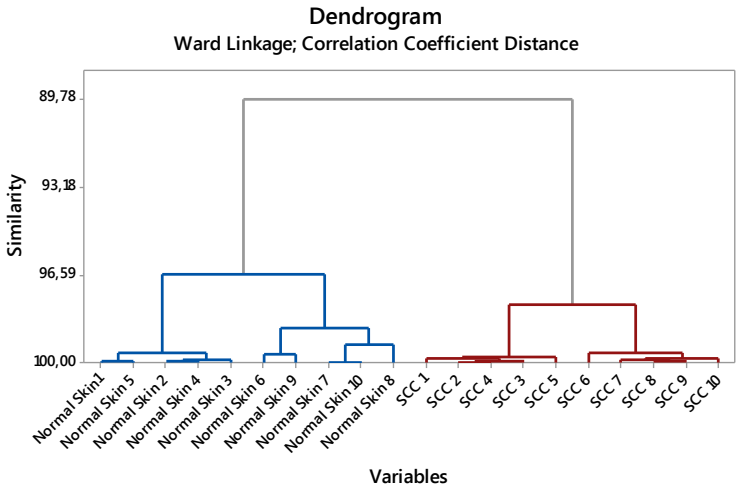

Figure 2. HCA for normal and SCC tissue samples

Figure 2 shows the graph obtained from the spectra of normal and SCC skin tissue and provides satisfactory separation between analyzed samples. On the basis of connecting distances, two distinctive groups were defined of both ten elements. The cluster in blue holds spectral data with 96.55% of similarity and is formed by normal skin tissue. The branches highlights the biological variability of the animal model used to develop the disease. The red

cluster is composed by SCC data and shows similarity of 97.76%, and we believe that the offshoots presented are related to different stages of the disease.

4. Conclusions

We conclude that FTIR spectroscopy associated with cluster analysis can differentiate samples of normal skin from SCC tissue even when these present high similarity with each other, highlighting the potential of the technique for diagnostic purposes.

5. References

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