THE USE OF RAMAN MICROSPECTROSCOPY FOR CHARACTERIZATION OF TUMOR AND TUMOR MARGIN CELL POPULATIONS

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INTRODUCTION

Squamous and basal cell carcinoma (SCC, BCC) are the most common cancers in the maxillofacial region. Finding new tools for a more reliable and timely diagnosis would be of substantial importance and would improve therapy outcome. Raman microspectroscopy has emerged as a promising technique for in vitro and in vivo, non-destructive detection and biochemical characterization of several types of cancer.

AIM

To compare Raman spectra of cells originating from oral squamous cell carcinoma (OSCC), basal cell carcinoma (BCC) and their surgical margins (SM) (Figure 1), with the aim of evaluating the ability of Raman spectroscopy to detect differences between the two types of neoplasms and their surgical margins, as well as between tumor cells and control cells.

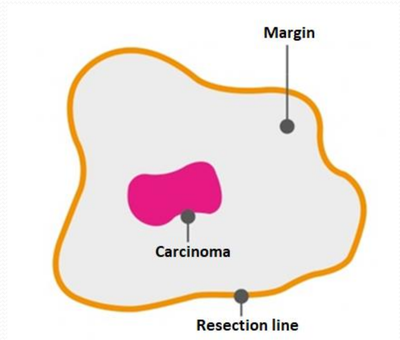


Figure 1. The relation between tumor tissue and its margin

METHODS

OSCC, BCC and SM cell cultures were generated. Control cells were generated from healthy skin of a patient undergoing benign tumor excision. The cells from the 3rd passage were used for the experiment (Figure 2). After passing and counting cells, 1x106 cells of each culture was separated in centrifuge tubes in culture medium. After centrifugation, the precipitate was transferred without fixation directly to a gold microscope plate for Raman microspectroscopy. Raman signals were obtained by the HORIBA Jobin Yvon Xplora spectromicroscopic apparatus (HORIBA Jobin Yvonne S.A.S., Villeneuve-d'Asck, France) equipped with microscope BKS51 (Olympus, Tokyo, Japan). Laser diode at a wavelength of 785 nm and power of 90 mV, magnification of 100x, focus size of 2 μm and a time exposure of 100s was used. CCD camera (Syncerity, HORIBA Scientific, Edison, New Jersey, USA) was used for spectrum recording. All cell samples were recorded 30 times, by random selection of points. Raman cell spectra were observed in the range of 400-2600 cm-1. Acquisition of Raman spectra was performed using LabSpec 6 software (HORIBA Jobin Yvon S.A.S., Villeneuve-d'Ascq, France).

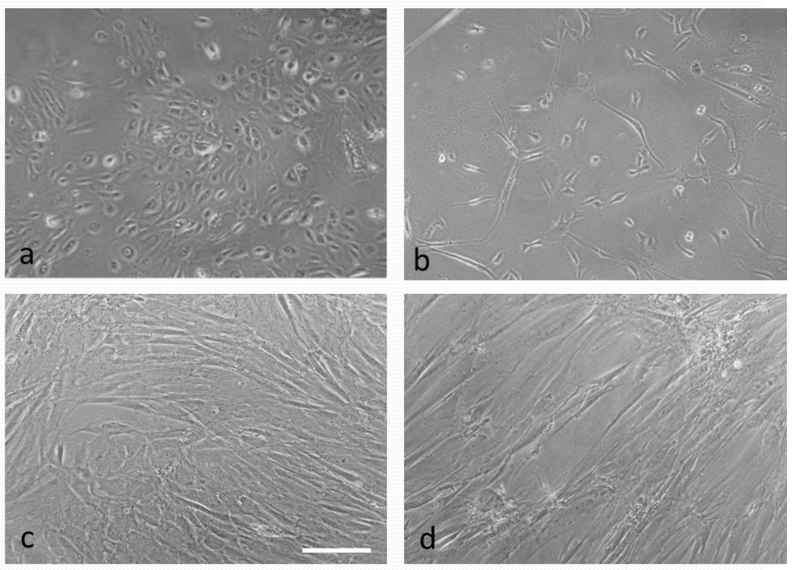


Figure 2. OSCC cells (a), OSCC margin cells (b), BCC cells (c), BCC margin cells (d)

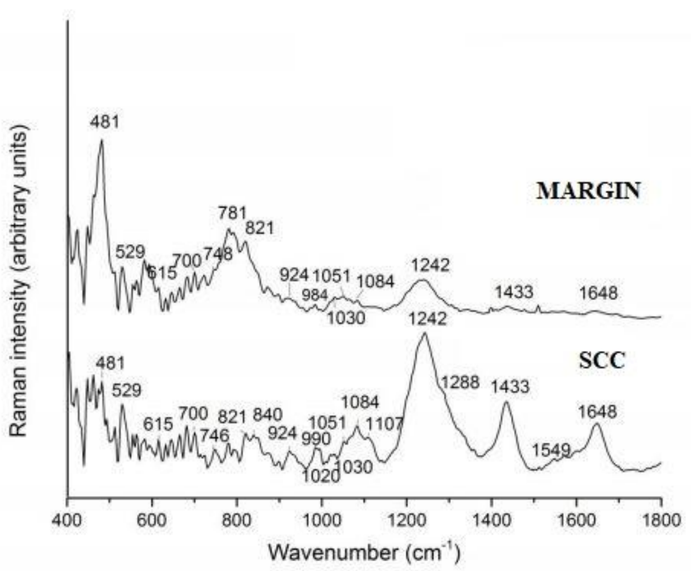
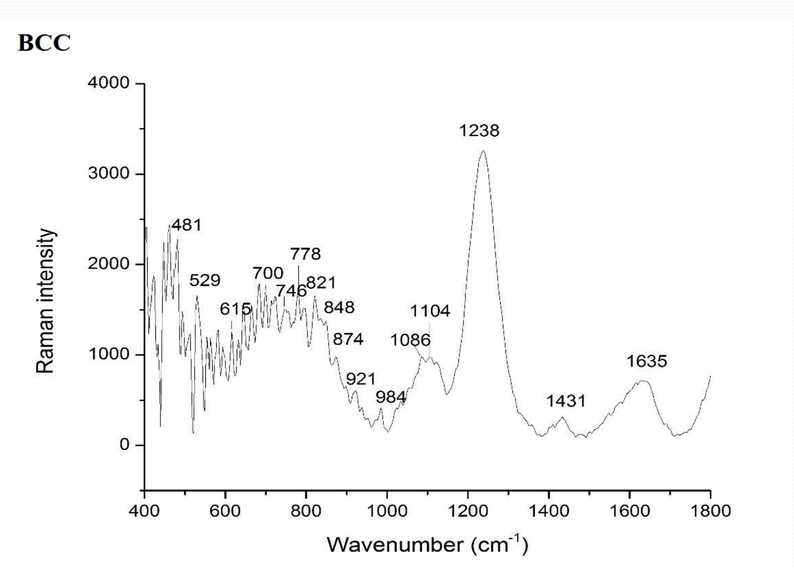


Figure 3. Raman spectra of OSCC and its margin



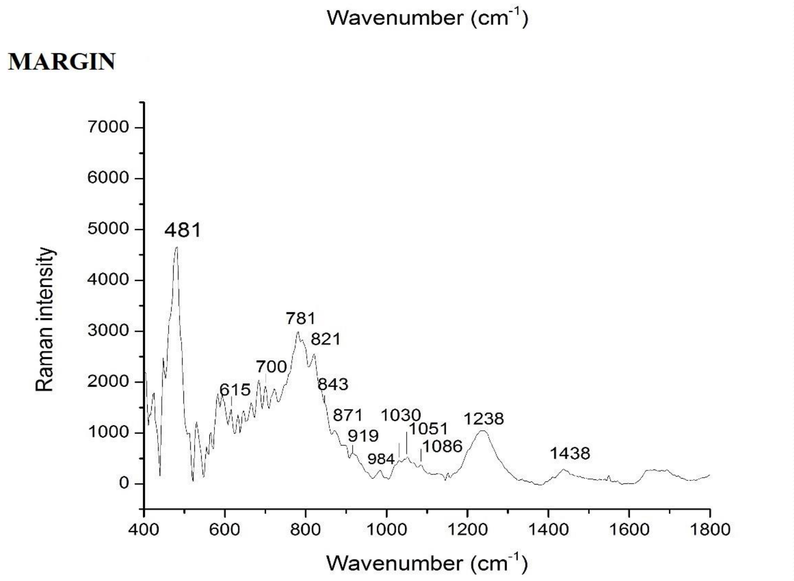


Figure 4. Raman spectra of BCC and its margin

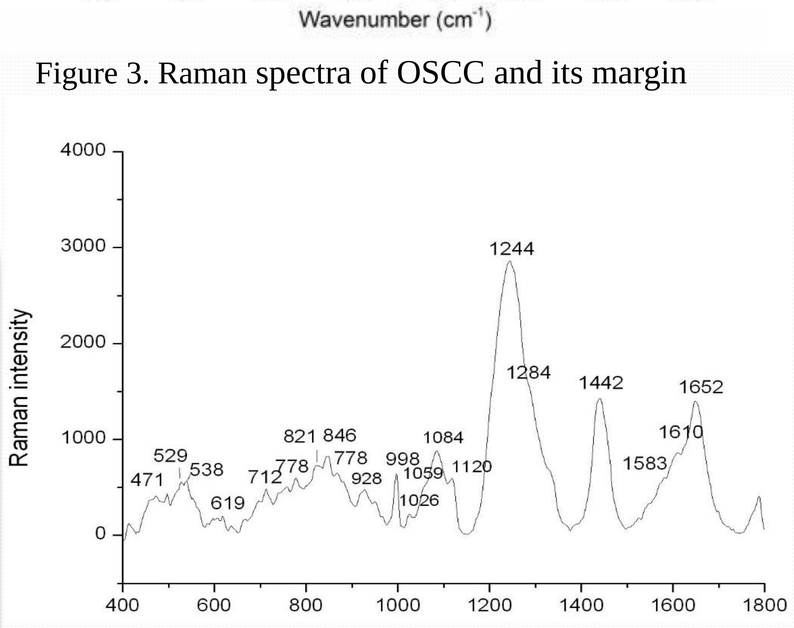


Figure 5. Raman spectra of control cells

RESULTS

The analysis of OSCC showed that amide peak (1242 cm-1) was more prominent in tumor cells than in margins; similarly, the amount of protein and lipids (peaks 1433 and 1648 cm-1) was higher in tumor cells than in margin cells. On the other hand, DNA / RNA levels (peak 781 cm-1) were higher in surgical margins (Figure 2).

Analysis of BCC Raman spectrum showed high levels of type I collagen, amino acids proline, hydroxyproline and valine (921-984 cm-1) as well as amide III (peak 1238 cm-1). The intensity of the 1635 cm-1 peak indicates an increased amount of protein and lipids in tumor cells compared to margin cells (Figure 3).

CONCLUSIONS

The analyzed tumor, tumor margin, and control cells displayed remarkable similarities, with however occasional differences sufficient to distinguish normal from cancer cells.