HR-MAS Spectroscopy of Oral Cancer Tissues and Their Assessment with Histopathological Examination

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INTRODUCTION

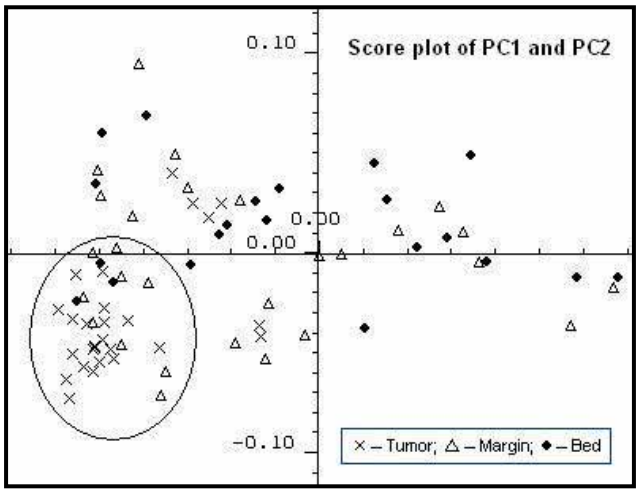
Oral squamous cell cancer is the most common form of malignancy in India at present. Unfortunately a large number of patients present in advanced stage, which is beyond curative treatment. Surgical excision with adequate margin is the treatment of choice in most patients. Adjuvant treatment in form of radiotherapy and chemotherapy is used to prevent or reduce the rate of recurrence. Adjuvant therapy is not without side effects and affects the quality of life. Adequate assessment is required to select patients who obtain maximum benefit. The assessment is traditionally by histopathology to obtain the status of tumor margins. High-risk patients with positive margins are offered adjuvant treatment but the rate of recurrence is still high and histologically negative margins may show tumor recurrence in the bed much to the dismay of the surgeon. Methods that can identify the molecular and DNA changes may provide the data needed to predict the true malignant potential of the lesions with a higher degree of certainty. With these objectives, we have evaluated the role of Proton HR-MAS Spectroscopy of the tumor margins and tumor beds by analyzing the metabolites, which can serve as markers for malignancy in tissue to improve our identification of high risk patients and selection for aggressive treatment, and to correlate our results with subsequent histopathological analysis on the same tissue specimen.

MATERIALS AND METHODS

The oral cancer tissues (about 100 mg wet weight) from tumor, margin and bed (n=107) were snap-frozen in liquid nitrogen from patients (n=24) undergoing surgery, and were stored in liquid nitrogen until NMR experiments were performed. Prior to NMR analysis, the tissues were thawed and 30-40 mg of wet weight tissue was dissected from the oral cancer sample for HR-MAS NMR analysis. The HR-MAS experiments were performed on a Bruker BioSpin *Avance* 400 MHz FT-NMR spectrometer using $^{1}H/^{13}C$-Dual 4 mm HR-MAS probehead with Z-shielded gradient at Magic Angle. One-dimensional ^{1}H NMR were performed using NOESY pulse-sequence with water-presaturation during relaxation delay of 2 s and a repetition time of 4 s at 25°C. One-dimensional CPMG experiments were also recorded for all the samples. All the experiments were performed with a spinning speed of 4 kHz. On few samples, two-dimensional COSY and HSQC using adiabatic pulses were also performed for resonance specific assignments. The NMR data were further subjected to chemometric Principal Component Analysis (PCA). All the analyses were done by Bruker's software program AMIX 3.5.

RESULTS

The spectra generally comprised of fatty acids, triglycerides, along with presence of long T_2 components such as valine, leucine, isoleucine, lactate, alanine, glutamate, creatine, taurine, choline, α-glycerophosphoryl choline and *myo*-inositol. The confirmation of all these metabolites were further carried out by the combined use of 2D COSY and HSQC spectra. All the spectra from region 2.5-4.5 ppm were subjected to PCA so as to define important descriptors for differentiation of oral tissue samples of tumors, margins and beds. The PC1 Vs PC2 score-plot is shown in the Figure, which clearly discriminated the cluster (encircled) of malignant tissue samples.



The buckets of signals arising due to choline, creatine, *myo*-inositol and the methine of lactate were the main discriminants for the observation of this cluster indicating malignancy. All these tumor specimens were found to be histologically malignant; whereas, seven histo-benign tissue samples from the margins and three from beds were also found to be malignant in nature (9.3%). Out of six tumor tissues outside the encircled area, four were found to be histo-benign, and in two specimens few cells of squamous cell carcinoma were identified. Analysis of the respective NMR spectra of the two individual histo-malignant tissues revealed presence of strong concentration of fatty acids along with the metabolites mentioned above responsible for malignancy. The PCA provided a specificity of 98% when compared to the histopathological examinations.

DISCUSSION

The NMR spectra patterns of the malignant tissues were completely different from the benign ones. The proton HR-MAS spectroscopy has the potential to discriminate malignancy of those oral tissues that are clinically, radiologically and histopathologically found to be negative. To the best of our knowledge, it is a first proton NMR spectroscopic report for evaluation of the resected margin and left bed in oral cancer. It can lead to valuable information, which can guide further therapy regarding post-surgery radiation and chemotherapy. Moreover, patients with negative molecular assessment may be spared adjuvant radiation. Patients with positive margins and bed as defined by tissue NMR in conjunction with PCA will benefit from more aggressive chemotherapeutic approach. We believe that the data presented has its potential utility in management of patients undergoing surgery for oral cancer.