

# Perineural invasion in Oral Squamous Cell Carcinoma tissues: HR MAS NMR study revisited

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**Introduction:** Oral Squamous Cell Carcinoma (SCC) represents more than 90% of all head and neck cancers with the worldwide incidence of more than 350,000 new cases per year (1). The histopathology, a gold standard technique cannot give explanation why certain tumors metastasize while other tumors grow to larger size and remain restricted. Therefore, newer methods are required for understanding of intracellular features of post-surgical tumor tissues and the extent of penetration of disease in tissues. In continuation to our earlier studies<sup>2</sup>, HR MAS NMR spectroscopic studies have been further performed on resected human (n= 48) oral Squamous Cell Carcinoma (SCC) biopsies, its neighboring margins including nerves, muscles, arteries, facial fibroadipose tissues and bed tissues (n=188) to understand the extent of perineural invasion of cancer.

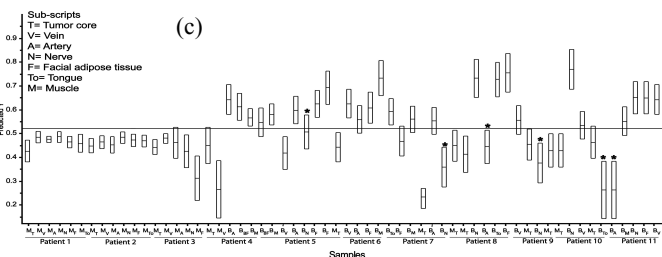
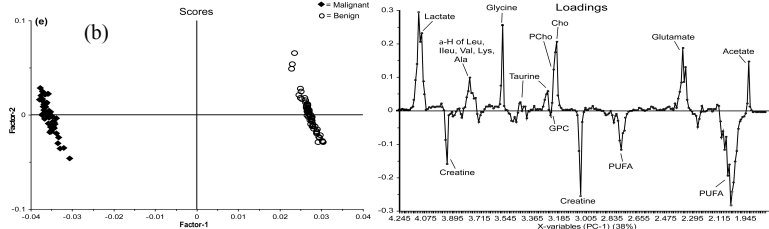
**Materials and Methods:** Tissue specimens (n=188) were obtained from 48 patients (35 males and 13 females) who were enrolled with a written consent to participate in the study that was approved by the ethics committee. The stored tissues at -80°C were thawed and washed with D<sub>2</sub>O prior to NMR analysis, to remove blood and other impurities. The tissues were then dissected and the inner-core (30-40 mg of wet-weight) of the tissues was taken for HR-MAS NMR experiments. Typically, the sample was packed into a 4 mm ZrO<sub>2</sub> rotor of 50 µl capacity; a volume of 20µl of D<sub>2</sub>O having 0.03% TSP was used as a chemical shift reference. The sample-rotor-setup was then transferred into the HR-MAS NMR probe for NMR analysis on Bruker Avance 400 MHz NMR spectrometer. Proton NMR spectra with water suppression were acquired using one-dimensional NOESY CPMG spectra at 8.0 °C. The data from 37 patients (128 tissue specimens) were subjected for training set for OSC-filtered PLS-DA model using the software 'The Unscrambler X' Software package (Version 10.0.1, Camo ASA, Norway) and the rest eleven patients tissue specimens (n=60) were classified according to the model.

**Results and Discussion:** The OSC-filtered PCA model indicated that malignant tissues had higher levels of glutamate, choline, phosphocholine, lactate, acetate, taurine, glycine, leucine, lysine, isoleucine and alanine, and lower levels of creatine and PUFA, representing altered metabolic processes (lipidogenesis, protein synthesis, and volume regulation) during tumor progression as observed in the PCA loading plot (Fig b). The regression coefficient OSC-filtered PLS-DA model was then generated for classification of unknown sample. The unknown tissue specimen's proton HR-MAS spectra were correctly classified in its respective histological categories with

>90% of correct classification and 100% sensitivity and 82.5% specificity. The overall representation of metabolic shift in this prospective <sup>1</sup>H HR MAS NMR study goes hand-in-hand with the tumor biology, which is explained by the results of multivariate statistical analyses. In this study, nerves, arteries, veins, facial tissues and other neighboring tissues were also studied for determining the presence and absence of peri-neural invasion along with identification of affected facial adipose tissues and muscles involved.

**Conclusions:** The proton HR-MAS MR spectroscopy could efficiently identify the metabolic derangements of malignant tumor from non- malignant tissues and seems to be promising in understanding the extent of tumor penetration in neighboring malignant nerves, arteries and marginal tissue specimens. However larger sample size is required for such endeavour but the metabolic profile may be utilized in future for real time diagnostics to monitor surgical patients due to the technical simplicity of HR-MAS NMR spectroscopy.

Figure 1:(a) <sup>1</sup>H CPMG NMR spectra of malignant and benign tissues and histopathology report of the malignant tissue specimen as observed in our earlier study<sup>2</sup> (b) PCA score and its corresponding loading plot of tissue biopsies obtained from bladder cancer patients indicating the variations of resonances among malignant and non-malignant tissues and (c) prediction plot of unknown



## References:

- [1] Hermans, R. (2005). Oropharyngeal cancer. *Cancer Imaging*, 5(Spec 793)
- [2] Srivastava et al *Metabolomics* (2011). 7:278–288.