Metabolic profiling of Urinary Bladder Carcinoma Tissues by HRMAS NMR spectroscopy

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Introduction: Urinary bladder cancer is a major epidemiological problem that continues to grow each year, therefore, its early detection and diagnosis reduces the mortality rate and so the morbidity. Urinary bladder cancer (UBC) is the seventh most prevalent type of cancer worldwide. Approximately fifty percent incidences of bladder cancer occur due to cigarette smoking and the remaining large portion, suffers due to their exposure to numerous industrial and/or agricultural carcinogens. These carcinogens induce multiple genetic changes which result in morphological changes and altered cellular metabolism in urothelium. In recent times, high resolution ^{1}H NMR and MRS based metabonomic studies have successfully provided metabolic insight in diseased states. It is well documented that NMR spectroscopy gives non-invasive, qualitative and quantitative information regarding the metabolite in the form of signals which are specific for each single metabolite. These potential advantages of ^{1}H NMR spectroscopy in disease diagnosis have prompted us to analyze tissue biopsies and urine of patients suffering from non-muscle invasive bladder cancer with the aim of obtaining information about significant alterations in metabolic composition of tissues of bladder cancer patients.

Material and methods: The current study is a prospective correlation between ex-vivo HR MAS spectroscopy and in vitro NMR spectroscopy of voided urine with histopathology in superficial urinary bladder cancer. Tissue specimens s (n =53) were obtained from 24 patients who were enrolled with a written consent to participate in the study that was approved by the ethics committee at King George's Medical University, Lucknow. Tissue samples comprising of tumor and mucosa of urinary bladder (which seemed normal) with pre-operative urine sample of same patient were obtained from each patient. All these tissue samples were stored in high quality plastic vials and snap-frozen in liquid Nitrogen at the time of surgery, to stop all the enzymatic and consequent metabolic activities and were then stored at −80°C till the NMR experiments were performed. The NMR experiments were performed at 8^{0}C on a Bruker Fallandan Switzerland 400 MHz FT NMR spectrometer equipped with a 4mm ^{1}H/^{13}C HR-MAS dual probehead. After spectroscopic analysis, the tissue samples were initially fixed in 10% formalin, and were further embedded using paraffin. Sections of 5 mm at 100 mm intervals were taken to include the whole tissue specimen and were stained using haematoxylin and eosin. Sections were assessed whether or not a tissue specimen proved to be malignant. Overall 24 tissue specimens, after surgery, were identified to have cancer.

Results:

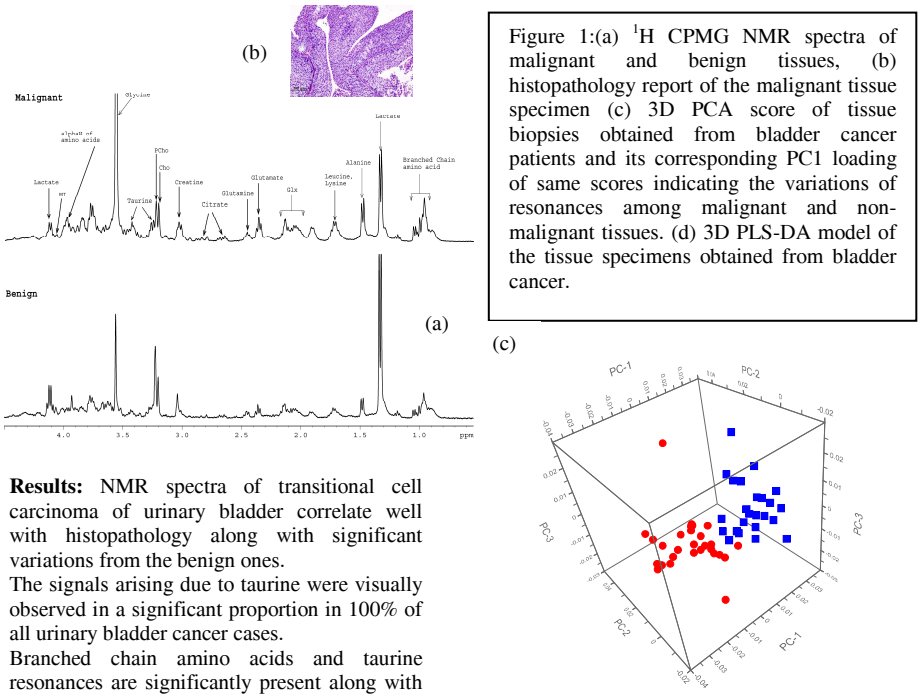
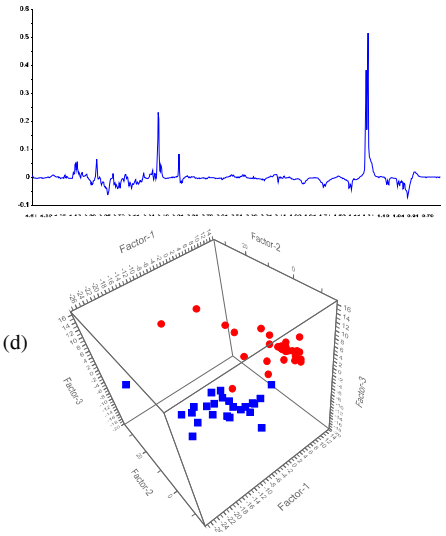


Figure 1:(a) ^{1}H CPMG NMR spectra of malignant and benign tissues, (b) histopathology report of the malignant tissue specimen (c) 3D PCA score of tissue biopsies obtained from bladder cancer patients and its corresponding PC1 loading of same scores indicating the variations of resonances among malignant and non-malignant tissues. (d) 3D PLS-DA model of the tissue specimens obtained from bladder cancer.



Results: NMR spectra of transitional cell carcinoma of urinary bladder correlate well with histopathology along with significant variations from the benign ones.

The signals arising due to taurine were visually observed in a significant proportion in 100% of all urinary bladder cancer cases.

Branched chain amino acids and taurine resonances are significantly present along with other metabolites as shown in figure1 (c) loading plot. The validated PLS-DA model showed >85% of correct classification (Q^2).

Discussion: As a consequence of various mutations, modifications in gene expression occur resulting in altered cellular protein composition and their activity, ending up in affecting the cellular and tissue metabolic fluxes. These modifications result into altered biochemical balance of metabolites in cancer cells and surrounding fluid. The rapid cell proliferation needs to complete a round of cell division which necessitates a cell grow and copy its DNA content. Both these process requires nutrients, energy and higher biosynthetic activity to duplicate all macromolecular components during each passage through cell-cycle while maintaining the cancer cell homeostasis. Both these processes require nutrients, energy and higher biosynthetic activity to duplicate all needs to complete a round of cell division which requires a cell to grow and copy its DNA contents.

Therefore, cellular processes occurring in malignant cells are quite different from those of non-proliferating cells and these metabolic perturbations reflect deviations occurring in biologic condition of disease. Whereas the branched chain amino acids are proteinogenic in nature, choline-containing compounds play an important role in cell proliferation. Taurine being reported as a urinary biomarker in our earlier study[2] was also found to be present significantly in malignant tissue specimens as well. However larger sample size is required for such endeavour but the biochemical information thus obtained may be moulded into an effective model parallel to histopathology.

References:

[1] M. E. Mycielska et al *Bioessays* **31** (2009), 10-20.
[2] Srivastava et al *Cancer Biomarkers* **6** (2010), 11-20