1H HRMAS NMR based metabolomics of benign and malignant neuro-endocrine tumors and its comparision with oral cancer and benign gall bladder tissues

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INTRODUCTION:
Medical diagnosis and treatment efficacy will improve significantly when a more personalized system for cancer assessment is implemented. Achieving this goal will require a comprehensive metabolic profiling of individuals suffering from various kinds of malignant and non-malignant tumors. In recent times, high resolution ^{1}H NMR based metabonomic studies in tissues have proved its metal in disease diagnosis.^{1}H HRMAS NMR spectroscopy of tissues combined with multivariate pattern recognition techniques provide qualitative and quantitative information about small metabolites in one step. These potential advantages of ^{1}H NMR spectroscopy have been exploited for defining the biochemical perturbations occurring in cancerous and non-cancerous tissues. In the present study, metabolic profiling of malignant and benign tumors of neuro-endocrine system (brain and thyroid) has been done. Further, these tumors are compared with oral SCC (squamous cell carcinoma) and benign gall bladder tissues, for comprehensively defining and distinguishing the metabolic requirements of neuro-endocrine system (for its proper functioning) with respect to other tissues of the body.

MATERIALS AND METHODS:
The tissue samples were collected in ependorff tubes from patients of schwannoma, meningioma, astrocytoma, glioblastoma, oral squamous cell carcinoma, thyroid tumor and gall bladder tissues (calculous chronic cholecystitis), after surgical removal of tumor tissues. The tissues samples were snap frozen in liquid nitrogen immediately after collection and were then stored in -80°C, until NMR spectroscopic analysis. Typically, the sample was packed into a 4 mm ZrO_2 rotor of 50 μl capacity; a volume of 20 μl of D_2O having 0.03% TSP was used as a chemical shift reference. The proton and CPMG HR-MAS spectra were recorded on a Bruker Avance 400 MHz spectrometer equipped with a 4 mm HR-MAS [1]H-^{13}C dual probehead with magic-angle gradient, operating at a proton frequency of 400.13 MHz. In all NMR experiments, the samples were spun at 4.0 kHz. The CPMG spectra of all the samples were analyzed using AMIX software (version 3.7.10, Bruker BioSpin, Switzerland). All spectra (n=115) were initially reduced to continuous integral segments (bins) of equal width (0.05 ppm each) and then statistically analyzed by principal component analysis (PCA).

RESULTS:
The present work has been undertaken with the aim of correlating: (i) spectral metabolite profiles with tissue pathologies and (ii) the impact of pathology on the function of the cell. Since pathological processes are likely to induce simultaneous changes on various metabolites, alteration in the level of a single metabolite may not provide the required insight regarding the biochemical processes occurring at molecular level. Hence, the ensemble of CPMG spectra was subjected to PCA to reduce the spectral complexity and for pattern recognition of metabolic variations. Typical ^{1}H HRMAS NMR spectra of benign and cancerous tissues are shown in Figure 1. The PCA score plot and its loading plot are represented in Figure 2. The principal components distinguishing malignant brain tumors (glioblastoma and astrocytoma) and oral SCC from non-malignant ones are creatine, glutamate, glutamine, GABA, alanine, taurine, glycine, phosphocholine and choline. The non-malignant tumors of brain and thyroid showed higher levels of myo-inositol, taurine and glycine and choline and significant increase in level of N-acetyl resonances of glycoproteins, scyllo-inositol and alanine in gall bladder tissues have defined their unique metabolic processes.
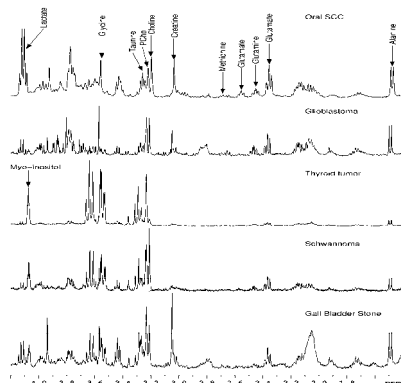

Figure 1: ^{1}H HRMAS NMR spectra of gall bladder tumors, schwannoma, thyroid, glioblastoma and oral SCC, depicting metabolic profile of each kind of tumor.
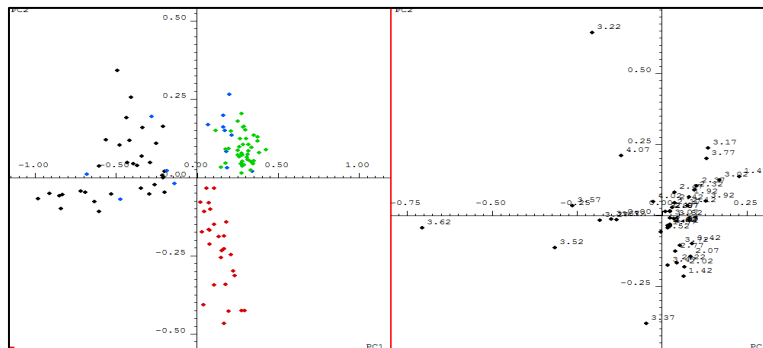

Figure 2: PCA score plot and corresponding loading plot depicts the contribution of small metabolites in distinguishing the benign and malignant tissues of neuro-endocrine system and the similarity between malignant brain tumors and oral SCC. The gall bladder tissues show a very different metabolic profile from rest of tumors. ●=gall bladder tissues (n=27); ●=Oral SCC (n=41); ●=brain tumor (n=14); ●=thyroid tumor (n=32).

DISCUSSION & CONCLUSIONS:
The contribution of alanine, glutamate, glutamine, choline, creatine, taurine and N-acetyl resonances of glycoproteins in different type of tissues provide a biochemical insight in the metabolic perturbations occurring in malignant and non-malignant neuro-endocrine tumors as well as in non neuro-endocrine tumors (oral SCC and gall bladder stone). This study demonstrates ^{1}H HRMAS NMR based metabolic profiling of various tumors for defining mechanisms critical to cellular function. Alanine is made by transamination of pyruvate to prevent increase in lactate content in the cells. Lactate is an end product of glycolysis in tumor tissues, which increases in tissues with hypoxia. Apart from being involved in osmoregulation and volume regulation, myo-inositol acts as a signalling and secondary messenger molecule in various biological processes like nerve guidance and intracellular calcium concentration control[1]. The altered levels of myo-inositol, choline, taurine and glycine, altogether explain the proliferation and volume regulation processes occurring in tumor tissues of schwannoma and thyroid tumors. On the other hand, glutamine, glutamate, creatine, choline, cis-aconitate and alanine explain the rapid cell proliferation, DNA and protein synthesis[2] alongwith cellular energetics of cancer cell metabolism in both malignant brain tumors and oral SCC. The higher N-acetyl resonances of glycoproteins explain the gall bladder stone pathology[3]. Thus, ^{1}H HRMAS NMR spectroscopy provides a promising approach to identify the key metabolites and their corresponding pathways that could be used in the monitoring of tumor progression and anti-cancer therapies.

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