Biochemistry of Soft Tissue Tumors: An in vitro NMR Study

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INTRODUCTION

Soft tissue sarcomas are diverse group of mesodermal tumors with highly aggressive biologic behavior. Though inherently aggressive, the morbidity and mortality is due to inadequate and improper treatment. The recurrent local failure and high systemic relapse are the main concern of these tumors. Presently the grade, size and depth of sarcomas are the most important factors used to estimate the risk of relapse and overall survival. But assigning a pathologic grade to an individual sarcoma as a means of predicting clinical behavior is often challenging, with a discordance rate as high as 30-40 %, even among expert and experienced pathologists [1]. This emphasizes the need for an objective and accurate assessment of sarcoma histologic type and grade, which could improve prognostication. There is a lack of information on the biochemical determinants of sarcoma proliferation and differentiation. MR spectroscopy detects metastases based on specific biochemical changes, which occur consistently with metastases, like elevated choline containing compounds, lactate and alanine etc. [2,3]. In the present study, we evaluated in a clinical setting the potential of in vitro MR spectroscopy in understanding the specific biochemical changes related to soft tissue tumors in comparison to normal tissue.

MATERIAL AND METHODS

Nineteen patients (patient characteristics are presented in Table 1) with soft tissue tumors were recruited in this study. The study was approved by Institute ethics committee and written informed consent was obtained from each patient. Patients underwent surgery and malignant and normal tissue were obtained and divided in two parts, of which one was immediately frozen in liquid nitrogen for NMR spectroscopic analysis and the other half was sent for histopathological evaluation. The perchloric acid extracts of the malignant and healthy tissue were prepared using the standard protocol and lyophilized powder obtained was dissolved in 0.6 ml of D_2O solvent. Sodium trimethyl silyl- (2,2,3,3-H4) propionate (TSP) was added as a standard for chemical shift and quantification of concentrations of metabolites. 1D proton spectra with water suppression were acquired with a relaxation delay of 14 seconds using DRX-400 (BRUKER, Switzerland). The concentration of metabolites were determined by comparing the integrated intensity of isolated resonances of the compounds of interest with that of the TSP signal. Various 2D NMR experiments were performed to assign the metabolic resonances. Student t test was performed to test the significance of results observed.

RESULTS

Table 1 presents the patient characteristics. In all 40 metabolites comprising of amino acids [(glutamic acid (Glu), glutamine (Gln), alanine (Ala), etc.), sugars [(Glucose (Glc) and myoinositol (mI)], membrane metabolites [glycerophosphorylcholine) (GPC), choline (Cho)], organic acids [(lactate (lac), fumarate, etc.,)] and nucleotides were unambiguously assigned using 2D NMR. The concentration of 9 metabolites was determined and presented in Table 2. Significantly higher concentration of lac, Ala, Cho, GPC, Glu + Gln, formate and fumarate in tumor was observed compared to normal tissue while no significant difference was observed in the concentration of α-ketoglutarate and acetate.

Table 1. Patient Characteristics.

No. of patients	19
Patient Age (y), Sex	14 male and 5 females, mean age 37 ± 18 yrs, age range 18-70 yrs
Tumor grade	High grade; n = 14, Low grade; n=5
Tumor size (Long axis in cm)	5-30 cm
Histopathological evaluation	Malignant fibrous histiocytoma-1, angiosarcoma-1, synovial sarcoma – 8, myxoid sarcoma-1, malignant peripheral nerve sheath tumor-1, leiomyoma-2, dermato fibro sarcoma -1, leiomyosarcoma -2, malignant mesenchymal tumor- 2

Table2. Concentration (mM/Kg wet weight, Mean ± SD) of metabolites in normal and tumor tissue.

Metabolites	Lactate	Alanine	Choline	GPC	Glu+Gln	Acetate	Formate	α-Ketoglutarate	Fumarate
Control	4.2±3.1*	1.3±0.8*	0.3±0.2*	0.4±0.3*	2.7±0.6*	0.9±0.6	0.5±0.4*	1.5±0.9	0.2±0.1*
Soft tissue Sarcoma	15.5±8.8	2.6±1.0	0.6±0.5	1.8±1.7	4.4±2.2	1.0±0.5	0.2±0.1	1.6±0.6	0.1±0.06

NOTE: *, $p < 0.05$ between control and soft tissue sarcoma.

DISCUSSION

To our knowledge, this is the first study delineating the comprehensive metabolite characteristics of soft tissue tumors in a clinical setting using in vitro NMR spectroscopy. Significantly higher concentration of Lac was observed in tumor tissue indicating higher rate of anaerobic glycolysis [2]. Higher concentration of membrane metabolites GPC and Cho may be attributed to the increased membrane synthesis in rapidly proliferating tumor cells [2]. Wang et al. documented that the Cho can be reliably detected in malignant bone and soft tissue tumors and can help in differentiation of malignant and benign tumors [3]. The biochemical changes in tissue lipid have been found to correlate with sarcoma cellularity, growth rate (mean mitotic activity) and differentiation [4]. Present study suggest that proton NMR spectroscopy analyses of soft tissue tumors provides clinically relevant biochemical signature which would be of value in determining prognosis and soft tissue sarcoma management.

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