

A Metabolomics Study of Necrosis in Sarcoma Using ^1H HR-MAS NMR Spectroscopy

J-H. Chen¹, R. B. O'Connor¹, P. DeCarolis¹, Y. V. Wu¹, R. C. Geha¹, S. Singer¹

¹Memorial Sloan-Kettering Cancer Center, New York, NY, United States

Introduction: Necrosis, the major cell death mechanism in tumors, can be induced by insufficient nutritional support due to rapid growth of the tumor or by chemo-/radio-therapy. The degree of necrosis is also an important prognostic factor for soft tissue sarcoma metastasis and recurrence¹. Traditionally, studies of tumor necrosis have been focused on tissue morphologic changes and biological mechanisms². A metabolomics study of necrosis in sarcomas using ^1H hr-MAS NMR spectroscopy was performed to elucidate the biochemical mechanisms involved in tissue necrosis and to identify metabolite markers that can distinguish necrotic from viable tissue in soft tissue sarcoma.

Methods: Viable and necrotic sarcomas from 17 patients were analyzed on a Bruker 600 MHz spectrometer at 20 °C using a MAS rate of 5000 Hz. An improved water-suppression technique was used to retain all the metabolites signals in the tissue sample³. Conventional pathologic analysis was done on the same set of samples after the NMR experiments. The metabolic differences between necrotic and viable tumors were analyzed. A supervised support vector machine (SVM) learning with principle components analysis (PCA) was applied to the complete set of ^1H spectra. Oil-Red-O staining identified fat droplet accumulation in the tumors.

Results: The lipid signal was 1.5-fold higher in necrotic compared to viable tissue. Fat droplet accumulation was detected by Oil-Red-O staining in necrotic but not viable tumor tissues. Small metabolites with the exception of lactate were reduced by more than 90% in necrotic compared to viable tissue. Finally, there was a greater than 90% reduction in protein amide proton signals in necrotic tissue. An SVM analysis confirmed these observations and was able to accurately discriminate the necrotic tumors from the viable tumors.

Conclusion: Tumor tissue necrosis was associated with a substantial loss in small metabolites and protein amide activity. The increased lipid signal in necrotic compared to viable tumor tissue results from fat droplet accumulation in necrotic tissue specimens. Necrotic tumor can be readily distinguished from viable tumors based on the ^1H hr-MAS NMR spectra profiles through quantitative analysis of low-molecular-weight metabolite, lipid and protein amide signals or by using SVM learning method. The substantial loss in protein amide proton signals provides an explanation for the prolonged water relaxation times detected in necrotic compared to viable tissue samples.

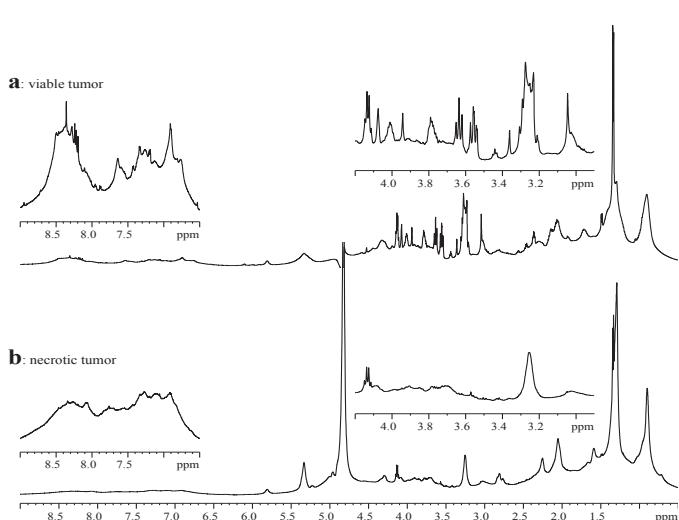


Fig. 1. ^1H spectra of representative tumors: **a)**. viable tumor; **b)**. necrotic tumor. The insets show the enhanced regions.

Reference:

1. Yeh, J. J. et al. Significance of necrosis and gene expression profiling in high-grade myxoid/round cell

liposarcoma (MRLS) of the extremity. *Journal of Clinical Oncology* 23, 819s-819s (2005).

2. Syntichaki, P. & Tavernarakis, N. Death by necrosis. Uncontrollable catastrophe, or is there order behind the chaos? *EMBO Rep* 3, 604-9 (2002).
3. Chen, J. H. et al. Water suppression without signal loss in HR-MAS ^1H NMR of cells and tissues. *J Magn Reson* 171, 143-50 (2004).