Published in final edited form as:

Adv Exp Med Biol. 2011 ; 701: 69–73. doi:10.1007/978-1-4419-7756-4_10.

³¹P-MRS Studies of Melanoma Xenografts with Different Metastatic Potential

Lin Z. Li,

Department of Radiology, School of Medicine, University of Pennsylvania, Philadelphia, PA19104, USA

Rong Zhou,

Department of Radiology, School of Medicine, University of Pennsylvania, Philadelphia, PA19104, USA

Dennis B. Leeper, and

Department of Radiation Oncology, School of Medicine, Thomas Jefferson University, Philadelphia, PA 19107, USA

Jerry D. Glickson

Department of Radiology, School of Medicine, University of Pennsylvania, Philadelphia, PA19104, USA

Abstract

Previously we reported that three imaging methods, dynamic contrast enhanced magnetic resonance imaging (DCE-MRI), $T_{1\rho}$ -MRI, and ultralow temperature NADH/flavoprotein fluorescence imaging (redox scanning), could differentiate the less metastatic human melanoma cell line A375P from a more metastatic line C8161 growing as mouse xenografts in nude mice (Li LZ *et al.* *Adv. Exp. Med. Biol.*, 2007, 599:67–78; PNAS, 2009, 106:6608–6613). The more metastatic C8161 tumor was characterized by less blood perfusion/permeability, a more oxidized mitochondrial redox state in the tumor core, and a smaller $T_{1\rho}$ relaxation time constant averaged across the entire tumor section. In the current study, we have further probed the bioenergetic status and tissue microenvironment of these tumors by applying whole tumor phosphorous magnetic resonance spectroscopy (^{31}P -MRS) to these two xenografts in a vertical bore 9.4-T Varian magnet. The phosphomonoester (PME)/ β NTP ratio and intracellular pH value (pH_i) were determined. The phosphomonoester (PME)/ β NTP was higher in the more metastatic C8161 tumors ($n=4$) than in the less metastatic A375P tumors ($n=4$) ($p < 0.1$). No significant difference between the pH_i of C8161 and A375P was observed.

1 Introduction

Identification of imaging biomarkers of tumor metastatic potential would facilitate treatment planning and aid in the design of agents that modify the tumor phenotype. For this purpose, we have used MRI and optical imaging to compare xenografts in athymic nude mice of the

most metastatic melanoma cell line (C8161) and the least metastatic cell line (A375P) in a panel of five human melanoma cell lines obtained from Dr. Mary Hendrix at Northwestern University. Three lines exhibiting intermediate levels of aggressiveness (A375M, A375P10, A375P5) were included in some of the optical and $T_{1\rho}$ -MRI experiments. DCE-MRI, $T_{1\rho}$ -MRI, and mitochondrial redox imaging (ultralow temperature NADH/flavoprotein fluorescence imaging) provided sensitive imaging biomarkers for differentiation of the two extreme phenotypes, i.e., most metastatic C8161 versus least metastatic A375P [1, 2]. In particular, the mitochondrial redox ratio ($Fp/(Fp+NADH)$) reflected the relative aggressiveness of all five human melanoma cell lines based on their *in vitro* ability to penetrate a Matrigel barrier in a Boyden chamber and the number of lung metastases measured *in vivo*. These data support the conclusion that the tumor microenvironment, including blood perfusion, oxygen/nutrition supply, as well as mitochondrial bioenergetic status of cancer cells, plays a key role in cancer progression to metastasis, which has also been supported by a number of studies in the literature [3–7]. The mitochondrion is the cellular power plant that generates ATP, the major high-energy fuel that drives many physiological processes including lipid metabolism and biosynthesis. Abnormalities in metabolic states and lipid metabolism have been associated with cancer progression to metastasis.

^{31}P -MRS, which measures the relative levels of Pi (inorganic phosphate), NTP (nucleoside tri-phosphate) and phospholipid metabolites such as PCr (phosphocreatine), PME (phosphomonoester) and PDE (phosphodiester), is useful for cancer diagnosis and prediction of treatment response in animal models and patients. For example, in patients with soft tissue sarcomas both PME/Pi and PDE/Pi ratios correlated inversely with the rate of pathological complete response, and the pretreatment PDE/PME ratio correlated directly with metastasis-free survival and overall survival [7]. In addition, ^{31}P -MRS measures intracellular pH on the basis of the relative chemical shift of inorganic phosphate referenced to α -NTP or PCr [8, 9].

The goal of the present study is to identify potential ^{31}P -MRS biomarkers of human melanoma metastatic potential. Our hypothesis is that the two human melanoma mouse xenografts (A375P and C8161), with widely different metastatic potential, will exhibit distinct differences that can be detected by ^{31}P -MRS. Our data demonstrate that the $PME/\beta NTP$ ratio can differentiate between these two tumor models ($p < 0.1$) while intracellular pH can not.

2 Materials and Methods

Melanoma cells (2×10^6) were subcutaneously implanted on the back, shoulders or in the thighs of 7–9 week old male athymic nude mice (NCI strain NCr-nu/nu). After 1–2 months, the tumors reached approximately 7–18 mm in the longest dimension, and the volumes ranged from 0.16 to 1.0 cm³, calculated by the tumor length L, width W and height H as measured by the calliper, i.e., $Vol = 1/6\pi L \times W \times H$. These tumors were then examined by ^{31}P -MRS in a vertical bore (89 mm) 9.4-T Varian NMR spectrometer. The mice were maintained under anaesthesia with 1% isoflurane in oxygen delivered through a nose cone. ^{31}P -MR spectra of two A375P and four C8161 tumors were obtained with a radiofrequency slotted

tube resonator (diameter ~13 mm) double-tuned to ^1H and ^{31}P . The phosphorous spectra of another two A375P tumors were obtained using a surface coil (diameter ~8 mm) also double-tuned to ^1H and ^{31}P to improve signal sensitivity for relatively smaller tumors. The magnetic field inhomogeneity was below 0.5 ppm following shimming on the proton signals. ^{31}P -MR spectra were acquired with a single pulse excitation with a TR (time of repetition) of 2 s and a bandwidth of 8000 Hz. Depending on the signal to noise ratio (SNR), the number of excitations ranged from 256–1028. The chemical shifts and relative intensities (peak areas) of PME, Pi, PCr, γNTP , αNTP , βNTP peaks were determined with NMR spectrum analysis software (NUTS) following proper phase adjustment and baseline correction. The intracellular pH values, pH_i , were quantified based on the shift of the Pi peak from the PCr peak using the Henderson-Hasselbalch equation.

3 Results and Discussion

Figure 1 shows typical phosphorous NMR spectra obtained for a C8161 and an A375P tumor. PME, Pi, PCr, γNTP , αNTP , βNTP peaks were clearly discernable in the spectra. Although field inhomogeneity could have been further improved by single voxel shimming to better resolve phosphorous peaks, we did not attempt to do this because significant tissue heterogeneity observed by MRI in the aggressive tumors [1] would keep the ^{31}P -MR peaks broad even with single voxel shimming. Table 1 includes means and standard deviations of the pH_i , the PME/ βNTP ratio, and the volume for the two melanomas (n=4 for each).

There was no statistically significant difference in the tumor volume between the two lines ($p > 0.1$). The average PME/ βNTP ratio was 0.9 ± 0.4 and 2.6 ± 1.4 for A375P melanoma and C8161 melanoma, respectively. The difference between the two lines had a moderate statistical significance ($p < 0.1$). Although more mice are needed to establish greater significance, the trend to higher PME/ βNTP ratios in the more aggressive C8161 tumors is in agreement with previous clinical [10] and non-Hodgkin's lymphoma patients evaluated by Arias-Mendoza *et al.* [11], which showed that these ratios increased with tumor grade.

The average pH_i of the more metastatic C8161 melanoma was less than that of the less metastatic A375P melanoma by about 0.2 pH units, which corresponds to a factor of 60% higher H^+ concentration in the more aggressive tumor. An acidic tissue microenvironment has been linked to malignancy and is believed to be one of the driving forces for cancer progression to metastasis [12–14]. However, the difference observed between the two lines here was not statistically significant. This could be due to the small number of tumors and/or possibly to contamination of NMR signals from muscles since the ^{31}P -MRS method used here was not volume selective. Still, this lack of significant difference could be explained by the fact that tumor acidity mainly exists in the extracellular space, and the pH measured on the basis of ^{31}P -MRS may mainly result from the contribution from the intracellular space. It is known that the pH in the extracellular space (6.3–7.0) of tumors in animal models is maintained acidic relative to the pH in the intracellular space (6.9–7.4), whereas, this pH gradient is reversed in normal tissues [14]. In the future, an extracellular pH indicator 3-aminopropylphosphonate (3-APP) will be injected to measure the extracellular pH by ^{31}P -MRS in order to test differences in the extracellular pH between these two melanoma xenografts.

One shortcoming of this preliminary study is that ^{31}P -MRS was conducted with-out volume selection. It is possible to have signal contamination from non-tumor tissues for relatively small tumors; although the contamination should be small and negligible for rather large tumors since the effective coil space for signal acquisition will be mainly filled up by tumor tissues.

In summary, this study has shown that ^{31}P -MRS should be able to differentiate between the metastatic and the indolent human melanoma mouse xenografts. In the future, volume selective ^{31}P -MRS with 3-APP can be used to probe the difference between tumors with different metastatic potential. The pH imaging using MR contrast agents could also be utilized to investigate the pH heterogeneity in tumor tissues [14].

Acknowledgement

The authors thank Dr. Mary Hendrix and Dr. Elisabeth Seftor at Northwestern University for providing melanoma cell lines. This study is supported in part by NIH Grant P01-CA56690-08 & 08S1 (PI: D.B. Leeper, Project Leader: J.D. Glickson) and P50-CA093372-04 (PI: M. Herlyn).

References

1. Li LZ, et al. Predicting melanoma metastatic potential by optical and magnetic resonance imaging. *Adv Exp Med Biol*, 2007 599: p. 67–78. [PubMed: 17727249]
2. Li LZ, et al. Quantitative magnetic resonance and optical imaging biomarkers of melanoma metastatic potential. *Proceedings of the National Academy of Sciences of the United States of America*, 2009 106(16): p. 6608–6613. [PubMed: 19366661]
3. Rofstad EK Microenvironment-induced cancer metastasis. *Int J Radiat Biol*, 2000 76(5): p. 589–605. [PubMed: 10866281]
4. Subarsky P and Hill RP The hypoxic tumour microenvironment and metastatic progression. *Clin Exp Metastasis*, 2003 20(3): p. 237–250. [PubMed: 12741682]
5. Gillies RJ, et al. MRI of the tumor microenvironment. *J Magn Reson Imaging*, 2002 16(4): p. 430–450. [PubMed: 12353258]
6. Vaupel P and Mayer A Hypoxia in cancer: significance and impact on clinical outcome. *Cancer and Metastasis Reviews*, 2007 26(2): p. 225–239. [PubMed: 17440684]
7. Dewhirst MW, et al. Relation between pO_2 , P-31 magnetic resonance spectroscopy parameters and treatment outcome in patients with high-grade soft tissue sarcomas treated with thermoradiotherapy. *International Journal of Radiation Oncology Biology Physics*, 2005 61(2): p. 480–491.
8. Bhujwalla ZM, et al. Estimations of intra- and extracellular volume and pH by P-31 magnetic resonance spectroscopy: effect of therapy on RIF-1 tumours. *British Journal of Cancer*, 1998 78(5): p. 606–611. [PubMed: 9744499]
9. Zhou R, et al. Intracellular acidification of human melanoma xenografts by the respiratory inhibitor m-iodobenzylguanidine plus hyperglycemia: A P-31 magnetic resonance spectroscopy study. *Cancer Research*, 2000 60(13): p. 3532–3536. [PubMed: 10910065]
10. Shukla-Dave A, et al. Prediction of treatment response of head and neck cancers with P-31 MR spectroscopy from pretreatment relative phosphomonoester levels. *Academic Radiology*, 2002 9(6): p. 688–694. [PubMed: 12061743]
11. Arias-Mendoza F, Smith MR, and Brown TR, Predicting treatment response in non-Hodgkin's lymphoma from the pretreatment tumor content of phosphoethanolamine plus phosphocholine. *Academic Radiology*, 2004 11(4): p. 368–376. [PubMed: 15109009]
12. Gatenby RA and Gillies RJ Why do cancers have high aerobic glycolysis? *Nature Reviews Cancer*, 2004 4(11): p. 891–899. [PubMed: 15516961]
13. Gatenby RA, et al. Acid-mediated tumor invasion: a multidisciplinary study. *Cancer Research*, 2006 66(10): p. 5216–5223. [PubMed: 16707446]

14. Gillies RJ, et al. PH imaging. IEEE Engineering in Medicine and Biology Magazine, 2004 23(5): p. 57–64.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 1. Typical ^{31}P -MRS spectra of an A375P (upper panel) and a C8161 tumor (lower panel). PME: phosphomonoester, Pi: phosphate; PCr: phosphocreatine; NTP: nucleotide triphosphate.

Table 1

Mean±standard deviation of the tumor volume, PME/ β NTP and pH_i in two melanoma lines differing in metastatic potential.

	Volume (cm ³)	PME/ β NTP	pH _i
A375P (n=4)	0.37±0.05	0.90±0.36	7.22±0.36
C8161 (n=4)	0.63±0.35	2.65±1.41	7.01±0.21
t-test p value	0.2	0.09	0.4