

EARLY DIAGNOSIS OF BRAIN TUMOURS THROUGH BIOFLUID METABOLOMICS

J.R. Larkin¹, A.M. Dickens¹, T.D.W. Claridge², D.C. Anthony³ and N.R. Sibson¹

¹*CRUK and MRC Oxford Institute for Radiation Oncology Department of Oncology, University of Oxford, Oxford, United Kingdom*

²*Department of Chemistry, University of Oxford, Oxford, United Kingdom*

³*Department of Pharmacology, University of Oxford, Oxford, United Kingdom*

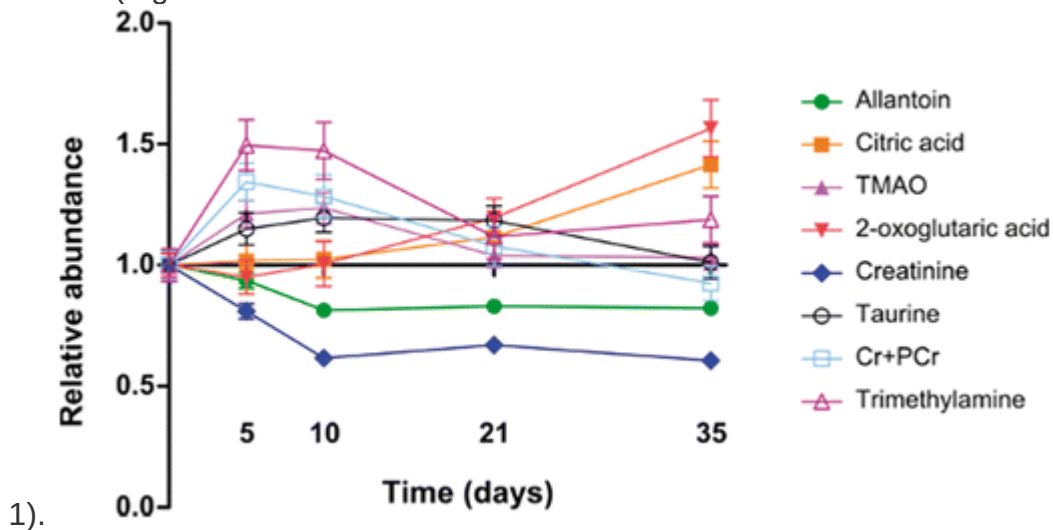
Abstract

Objectives: Over 20% of cancer patients develop brain metastases. Current MRI diagnostic techniques only detect late stage metastases, since they rely on blood-brain-barrier permeability to allow contrast enhancement. Thus, new methods enabling earlier diagnosis are urgently needed. We have previously shown that it is possible to discriminate between different inflammatory lesions in the CNS in rats (1), as well as between different stages of multiple sclerosis in patients (2), through biofluid (blood/urine) metabolomics. We believe that this ability is due, at least in part, to alterations in CNS metabolism, which provoke a specific metabolic signature in the biofluids studied. It is known that tumour metabolism differs markedly from normal brain and that brain metabolism itself will be altered by tumour presence. On the basis of the above, therefore, we hypothesised that the presence of brain metastases could be detected, *in vivo*, through NMR analysis of biofluids.

Methods: Metastatic mammary carcinoma cells (murine 4T1-GFP) were injected into BALB/c mice, via intracerebral, intracardial or intravenous routes to induce differing cerebral and systemic tumour burdens. Urine was collected at days 0 and 10 from all animals, and at days 5, 21 and 35 in animals injected intracerebrally. Samples from naïve, day 0 and vehicle-injected mice served as combined control cohorts. Urine metabolite composition was analysed using ¹H-NMR spectroscopy, and statistical pattern recognition and modelling was applied to identify spectral

differences and to identify commonalities indicative of brain metastasis burden. A robust method using unknown samples was used to validate model predictions.

Results: Significant metabolic profile separations were found between control cohorts and animals with tumour burdens at all timepoints for the intracerebral 4T1-GFP metastasis model ($q^2 = 0.59, 0.70, 0.81$ and 0.80 for days 5, 10, 21 and 35, respectively; $q^2 > 0.4$ considered significant). Models became stronger, with higher sensitivity and specificity, as the timecourse progressed indicating a more severe tumour burden. Sensitivity and specificity for predicting a blinded testing set were 0.89 and 0.82 , respectively, at day 5, but both rose to 1.00 at day 35. Key metabolites driving the separations were identified and quantified relative to control (Fig.



Significant separations were also found between control and day 10 animals for all 4T1-GFP injected mice irrespective of route ($q^2 = 0.70, 0.63$ and 0.78 for intracerebral, intracardiac and intravenous routes, respectively). The metabolites underpinning separations in each case differed indicating differentiation between systemic and CNS metastatic burden, but with common patterns that suggest a “fingerprint” for brain metastasis.

Conclusions: Our data indicate that animals with brain metastases can be identified using urinary metabolic profiles with NMR metabolomics, and differentiated from animals with a predominantly systemic metastasis burden. This approach may identify a set of biomarkers for the diagnosis of brain metastasis earlier than is currently possible, and may provide insight into metabolic disruption in brain metastasis.

References:

- (1) Griffin . (2004) FEBS Letters **568** 49-54; [Google Scholar](#)
- (2) Dickens and Larkin . (2014) Neurology **83** 1492-99 [Google Scholar](#)