The Metabolomics of Argininosuccinate Synthetase (ASS) Expression in Human Cancer by ^{1}H-NMR

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Abstract

ASS, the rate limiting enzyme for arginine synthesis, is aberrantly expressed in several human tumours. Arginine is an essential amino acid for tumours deficient in ASS, resulting in apoptosis following arginine deprivation. In contrast, arginine is non-essential for ASS (over)expressing cancers and may be utilised in a completely different way. Using an arginine lowering drug in patients with malignant mesothelioma (which is frequently deficient in ASS) we have analysed and validated the metabolic consequences of ASS expression and arginine utilisation in cancer cell lines using ^{1}H NMR.

Three batches of cell lines were selected:

(i) MSTO (ASS-negative) and H226 (ASS-positive)
(ii) JU77 (ASS-negative) and JU77 control
(iii) 2591 (ASS-negative) and 2591 control

The methods used for the extraction of metabolites from the selected cell cultures, and the preparation and measurement of the ^{1}H NMR spectra followed established protocols [1,2]. The cell lines were cultured and extracted at the Institute of Cancer and CR-UK Clinical Centre, Barts and the London. For each culture an aqueous extract, an organic extract, and a sample of the culture medium was prepared for NMR analysis. The ^{1}H NMR measurements were made at 600 MHz using the Bruker Avance 600 spectrometer at Queen Mary. In addition the measurements on the JU77 and 2591 batches were repeated at 700 MHz using the Bruker Avance 700 spectrometer at the Medical University of Lodz, Poland. These repeat measurements were made for cross-validation of the NMR method.

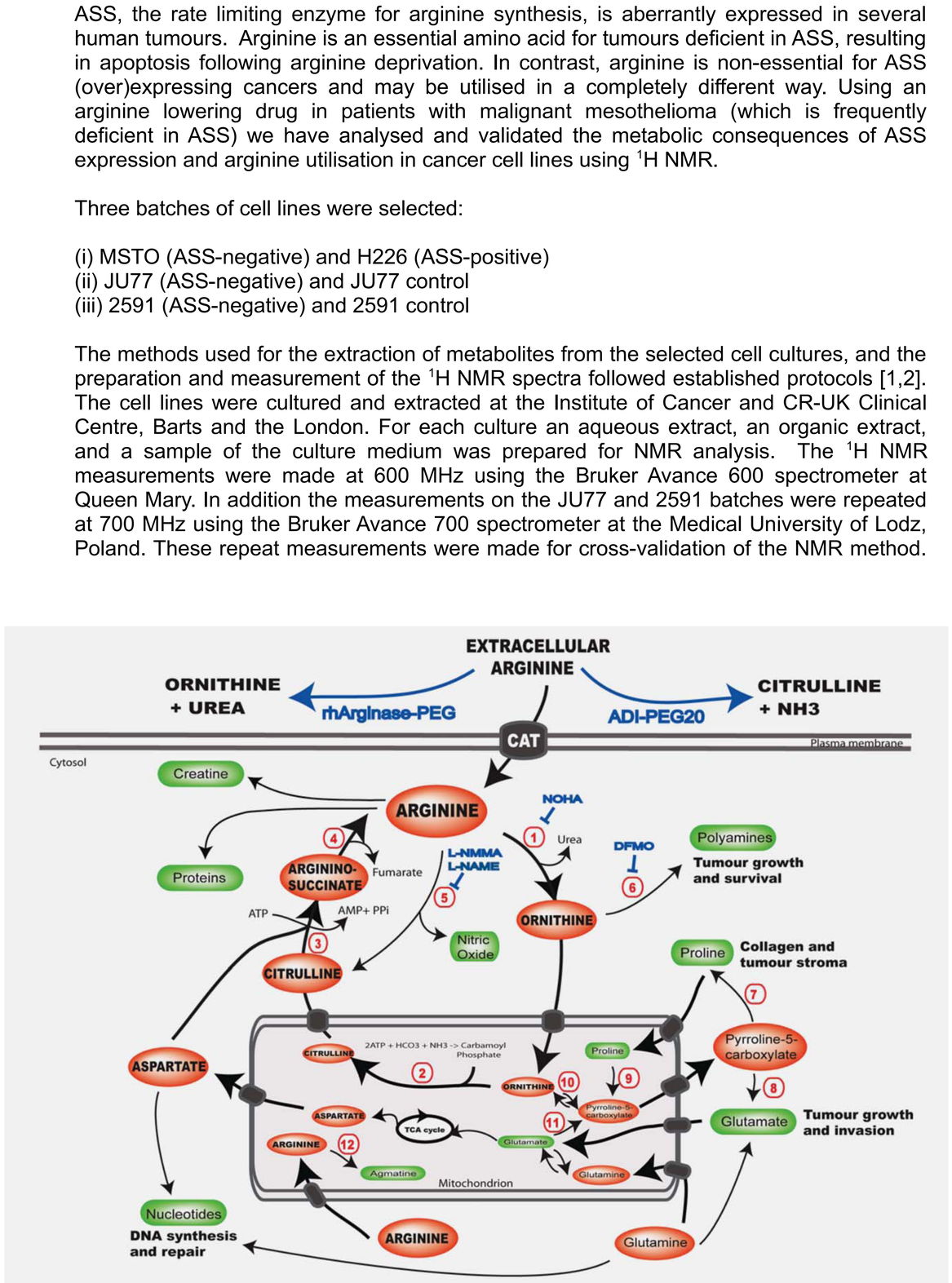

Figure 1. Arginine utilisation in cancer. Arginine is a substrate for a diverse array of metabolic and inflammatory pathways in health and disease. Arginine may be sourced via the cationic amino acid transporter (System y+), ASS1, or autophagy. Reprogramming of the arginine metabolome via inactivation or upregulation of ASS1 results in differential effects on tumourigenesis. The subcellular locations of key enzymes are shown and enumerated in the Figure as follow: 1, arginase 1; 2, ornithine transcarbamylase (OTC); 3, argininosuccinate synthetase (ASS1); 4, argininosuccinate lyase; 5, nitric oxide synthase; 6, ornithine decarboxylase; 7, pyrroline- 5-carboxylate reductase; 8, pyrroline-5-carboxylate dehydrogenase; 9, proline oxidase (dehydrogenase); 10, ornithine aminotransferase; 11, pyrroline-5-carboxylate synthase; 12, arginine decarboxylase. Several drugs affecting arginine bioavailability and metabolism are highlighted in blue: ADI-PEG 20, pegylated arginine deiminase; rhuArg-PEG, pegylated human arginase; DFMO, difluoromethylornithine; LNAME, N omega-nitro-L-Arginine methyl ester; L-NMMA, N-monomethyl-L-arginine; NOHA, N-hydroxy-L-arginine. In contrast to ADI-PEG 20, which is only active in ASS1-negative tumours, rhuArg-PEG has additional anti-tumour effects in ASS-positive liver cancer due to a specific lack of the urea cycle enzyme, OTC. In non-liver cells, arginine is re-generated via the citulline-NO cycle.

NMR data and statistical analysis

Data were imported and manipulated in Matlab (Mathworks) using in-house software written and compiled by Dr. T. M. D. Ebbels, Dr. H. C. Keun, Dr. J. T. Pearce, and Dr. R. Cavill. ^{1}H NMR spectra were automatically phased, baseline corrected, referenced to the TSP resonance at δ 0, and normalised to total intensity. For multivariate analysis the reduced resolution data were exported to SIMCA-P (Umetrics, USA). Principal Component Analysis (PCA) was applied to the reduced resolution data to reveal metabolic response.

The statistical total correlation spectroscopy (STOCSY) analysis method (3) was used to aid in the identification of potential biomarker molecules. STOCSY takes advantage of the multicollinearity of the intensity variables in a set of spectra to generate a pseudo-two-dimensional NMR spectrum that displays the correlation among the intensities of the various peaks across the whole sample. Supervised pattern recognition, orthogonal projection on latent structure-discriminant analysis (O-PLS-DA), was used to extract the part of the NMR spectra related to discrimination. This information was then cross-combined with the STOCSY results to help identify the molecules responsible for the metabolic variation (Table 1).

700M Hz

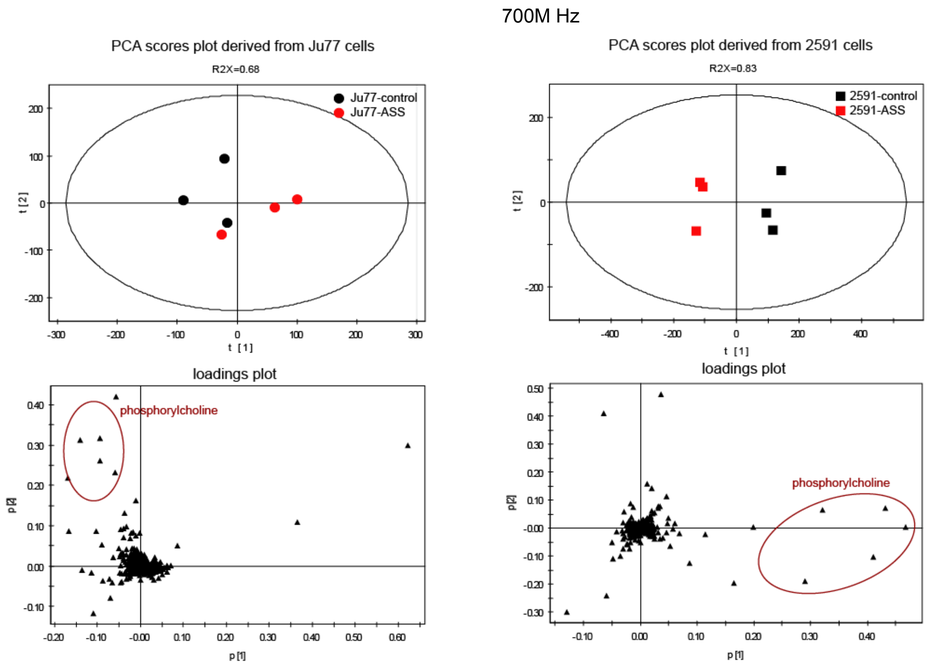

Figure 2. Scores and loadings plots for aqueous extracts from cell lines Ju77 and 2591 from 700 MHz data.

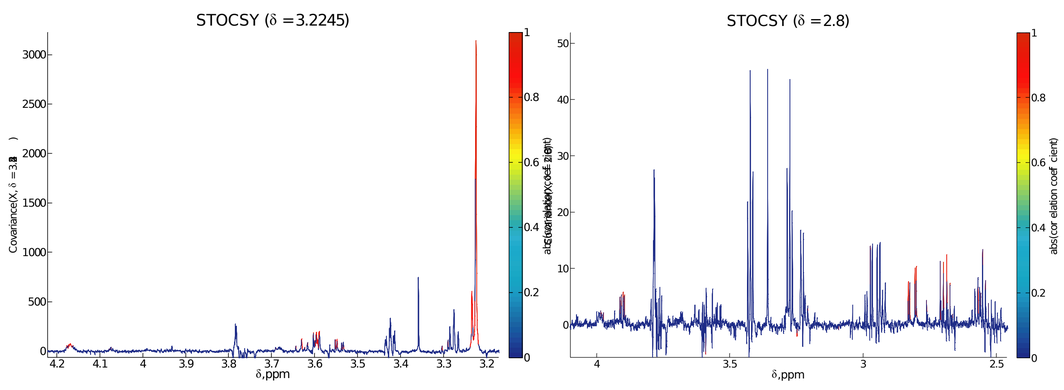

Figure 3. Projections from the STOCSY pseudo-two-dimensional spectrum taken at δ 2.8 (Aspartate) and δ 3.22 (Phosphorylcholine).

Metabolite	δ	JU77 600 MHz	JU77 700 MHz	2591 600 MHz	2591 700 MHz
Alanine	1.48	-28	-31	+25	+26
Aspartate	2.81 (2.69, 3.91)	(+50)	(+24)	224	+213
Choline	3.208	0	(-1)	0	+131
Phosphorylcholine	3.22 (3.6, 4.17)	-16	-16	-40	-40
Glycero-phosphorylcholine	3.235	0	(+2)	(-)	-36
Creatine	3.040	+265	+216	-35	-35
Creatinine	3.048	+415	+443	(+29)	+26
Glutamate	2.36 (3.78)	(+1)	0	+13	(+)
Glutathione (reduced)	2.56 (2.18, 2.94)	+36	(+)	+15	(+)
Lactate	1.33	-17	(-)	+10	(+)
Succinate	2.41	+39	+55	(-7)	(+9)
Taurine	3.43 (3.27)	+120	+140	+12	(+6)
Unknown 1	2.02	-23	(-)	-11	(-)
Unknown 2	2.06	-17	-15	+36	+40

Table 1. Percent change in metabolite in the ASS aqueous cell extracts, in comparison with controls. Values in parentheses are not so reliable as the corresponding signal intensities from the ASS cells overlapped significantly with those from controls. (-) and (+) signify a trend that cannot be quantified due to overlap.

References

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