Metabolomic Analysis of Renal Cell Carcinoma with High Resolution Magic Angle Spinning Magnetic Resonance Spectroscopy

Background

Renal cell carcinoma (RCC) is a metabolic disease, with the various subtypes exhibiting aberrations in different metabolic pathways. Metabolomics may offer greater sensitivity for revealing disease biology than evaluation of tissue morphology. In this study, we investigate the metabolomic profile of RCC using high resolution magic angle spinning (HRMAS) magnetic resonance spectroscopy (MRS).

Methods

Tissue samples were obtained from radical or partial nephrectomy specimens that were fresh frozen & stored at -80°C. Tissue HRMAS MRS was performed by a Bruker AVANCE spectrometer. Metabolomic profiles of RCC & adjacent benign renal tissue were compared, and false discovery rates (FDR) were used to account for multiple testing. Regions of interest (ROI) with FDR <0.05 were selected as potential predictors of malignancy. The Wilcoxon rank sum test was used to compare median MRS relative intensities for the candidate predictors. Logistic regression was used to determine odds ratios for risk of malignancy based on abundance of each metabolite.

Results

There were 38 RCC (16 clear cell, 11 papillary, 11 chromophobe) & 13 adjacent normal tissue specimens (matched pairs). Metabolic predictors of malignancy based on FDR include histidine, phenylalanine, phosphocholine, serine, phosphocreatine, creatine, glycerophosphocholine, valine, glycine, myo-inositol, scylla-inositol, taurine, glutamine, spermine, acetoacetate & lactate. Higher levels of spermine, histidine & phenylalanine at 3.15-3.13 ppm were associated with a decreased risk of RCC (OR $4x10^{-5}$, 95% CI $7.42x10^{-8}$, 0.02), while 2.84-2.82 ppm increased the risk of malignant pathology (OR 7158.67, 95% CI 6.3, $8.3x10^{6}$), and the specific metabolites characterizing this region remain to be identified. Tumor stage did not appear to affect the metabolomics of malignant tumors, suggesting that metabolites are more dependent on histologic subtype.

Conclusions

HRMAS-MRS identified many metabolites that may predict RCC. We demonstrated that those in the 3.14-3.13 ppm ROI were present in lower levels in RCC, while higher levels of metabolites in the 2.84-2.82 ppm ROI substantially increased the risk of RCC.

Table 1: Summary of metabolites that were significantly different between malignant and benign adjacent tissue, with odds ratios for risk of malignancy

	RCC (N=38)	Adjacent benign parenchyma (N=13)	P-value			
Age (years)	55.3 ± 11.4	50.8 ± 7.3	0.1818			
Males (n, %)	27 (71.1)	8 (61.5)	0.7302			
Race (n, %)	37 (97.4)	13 (100)	1.00			
Median MRS relative intensities (IQR)				**FDR P-value**	**Odds ratios (OR, 95% CI)**	**P-value for OR**
4.07-4.05 (Myo-Inositol)	0.80 (0.48, 1.32)	1.84 (1.27, 2.24)	0.0026	0.027	0.38 (0.18, 0.82)	0.013
4.02-4.00 (TBD)	1.21 (0.68, 2.07)	0.50 (0.06, 0.88)	0.0073	0.034	3.12 (1.10, 8.84)	0.032
3.99-3.96 (Histidine, Phenylalanine, Phosphocholine, Serine)	1.26 (0.84, 1.93)	2.56 (1.19, 3.50)	0.0092	0.013	0.34 (0.16, 0.71)	0.004
3.95-3.94 (Serine, Phosphocreatine)	0.77 (0.33, 1.24)	0.30 (0, 0.53)	0.0006	0.003	29.2 (2.47. 345.24)	0.007
3.93-3.91 (Creatine, Glycerophosphocholine)	1.28 (0.90, 1.61)	0.69 (0.24, 1.34)	0.0071	0.012	8.17 (1.77, 37.78)	0.007
3.61-3.59 (Myo-Inositol, Glycerophosphocholine, Phosphocholine, Valine)	0.96 (0.63, 1.24)	1.68 (1.39, 1.96)	0.0006	0.005	0.13 (0.03, 0.49)	0.003
3.55-3.52 (Glycine)	1.92 (0.77, 3.17)	4.02 (2.87, 4.42)	0.0019	0.024	0.59 (0.39, 0.90)	0.014
3.36-3.34 (Scylla-Inositol)	0.55 (0.35, 0.78)	1.34 (0.75, 1.54)	0.0019	0.005	0.08 (0.02, 0.42)	0.003
3.24-3.23 (Myo-Inositol, Taurine)	5.86 (3.95, 9.46)	4.32 (2.43, 5.40)	0.0267	0.030	1.35 (1.04, 1.76)	0.027
3.22-3.21 (Phosphocholine, Glycerophosphocholine, Histidine)	0.69 (0.22, 2.16)	4.23 (3.05, 5.53)	<0.001	<0.001	0.41 (0.35, 0.67)	<0.001
3.15-3.13 (Spermine, Histidine, Phenylalanine)	0.21 (0.11, 0.35)	0.83 (0.49, 1.02)	<0.001	<0.001	4×10^{-5} (7.42×10^{-8}, 0.02)	0.001
2.84-2.82 (TBD)	0.28 (0.18, 0.45)	0.18 (0.10, 0.23)	0.0021	0.009	7158.67 (6.3, 8.3×10^{6})	0.013
2.45-2.42 (Glutamine)	0.51 (0.30, 0.74)	0.32 (0.21, 0.38)	0.0098	0.017	121.5 (2.16, 6820)	0.02
2.15-2.11 (TBD)	1.45 (1.15, 1.97)	1.95 (1.46, 2.50)	0.0370	0.035	3.96 (1.18, 13.28)	0.026
1.93-1.92 (Acetoacetate)	0.31 (0.18, 0.67)	0.77 (0.54, 2.83)	0.0008	0.012	0.38 (0.13, 1.09)	0.072
1.35-1.33 (Lactate)	8.74 (5.26,13.23)	5.2 (3.06, 8.30)	0.0150	0.033	1.22 (1.03, 1.45)	0.023

*TBD denotes that the specific metabolites characterizing this region remain to be identified