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Studies of Colorectal Cancer by Nuclear Magnetic Resonance Spectroscopy

Review Article

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Abstract

Nuclear Magnetic Resonance (NMR) spectra have been recorded for urine samples of both male and female colorectal cancerous and healthy persons. Specific differences have been observed, in particular in the spectral range corresponding to some metabolites. The NMR-based metabolomics approach could help to better understand the mechanism of carcinogenesis and offer a non-invasive diagnosis tool.

Keywords: NMR Metabolomics; Urine Metabolomics; Colorectal Cancer.

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Introduction

The advancement of powerful physical methods allows the detection, analyses of several samples such as nanomaterials [1-8] and lived objects [9] etc…. These techniques must be used for the study of cancer, the most disturbing diseases of humanity for the present time [10-13]. Other approaches at the molecular level, such as metabolomics-based analytical techniques in Nuclear Magnetic Resonance (NMR) spectroscopy, Mass spectrometry (MS) may also be considered. In this paper, we shall present our study on colorectal cancer (CRC) by Nuclear Magnetic Resonance spectroscopy. Urine samples from CRC patients and healthy controls were analyzed with 1H-NMR spectra and their spectral profiles after subjection to multivariate analysis. We hope that the obtained results will furnish complementary data to those already obtained by other techniques, in particular by Laser Raman micro-spectroscopy [9-14] and should be usefull for the detection at early-stage CRC cancer by a non-invasive diagnosis. Some papers appeared already in recent years using NMR technique. Nevertheless if the sample is not animal (rat) [15], their number of samples was very limited [16] and did not suit to a statistical technique [17]. In this paper, we shall present our study with much larger number of samples of human urine.

Experiments

Urine samples were collected from 155 adults (86 males, 69 females, from 27 to 81 years, median age 45 years), including 77 CRC patients and 78 healthy persons, who have a physical examination in Medical Center MEDIC HCMC from June 2013 to July 2014. Diagnosis of colorectal cancer were made on the basis of usual clinical and laboratory findings and were confirmed by Colonoscopy. No patient has received chemotherapy, radiation therapy or surgery.

The collected urine samples were dissolved in D_2O with 0.1% 3 - (trimethylsilyl) - 1 - (propanesulfonate) TSP. The standard NOESY-1D NMR spectra were obtained on a Bruker AVANCE III 500 Ultrashield Plus Cryo NMR Spectrometer and their spectral profiles analyzed. Then, after normalization and scalling, Principal Component Analysis (PCA) and Partial Least Squares (PLS) were performed by Amix-version 3.9.14 – Bruker to reduce the dimensionality of the NMR data set for extracting useful information from complicated NMR data. The set of marker metabolites were identified from NMR data by Chenomx NMR suite 7.0 software.

Results and Discussion

Discrimination CRC spectra and Healthy spectra

The spectra of CRC and healthy persons are showed on Figure 1 where the principal peaks are assigned.

The assignment of principal peaks is based on the well known healthy urine spectrum already published by many authors [18-

Figure 1a. Aliphatic region 3.0 – 0.8 ppm on ^{1}H-NMR spectra of A: Healthy sample # 0022 - B: CRC sample # 1126.

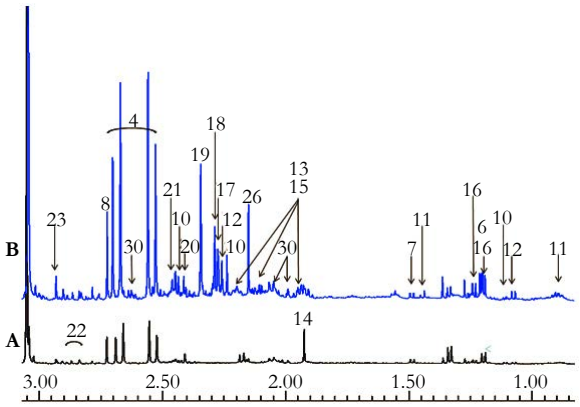


Figure 1b. Extended aliphatic region 4.3 – 3.0 ppm on ^{1}H-NMR spectra of A: Healthy sample # 0022 - B: CRC sample # 1126

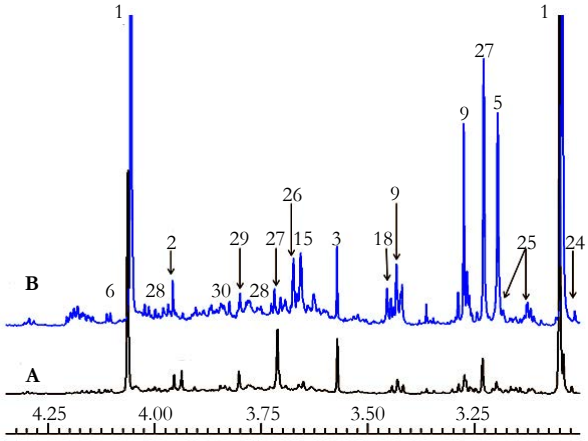
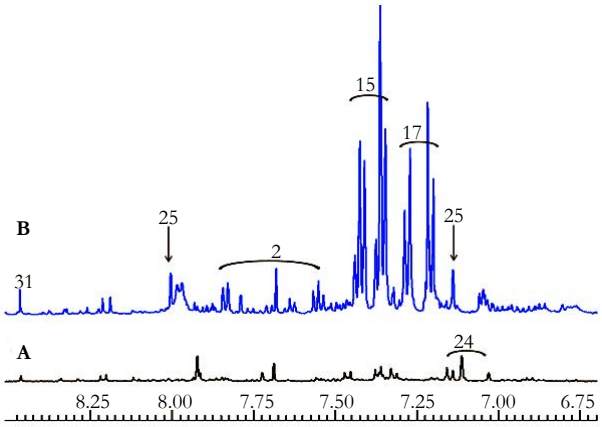


Figure 1c. Extended aliphatic region 8.5 – 6.6 ppm on ^{1}H-NMR spectra of A: Healthy sample # 0022 - B: CRC sample # 1126



21]:

1. Creatinine / Creatine; 2. Hippurate; 3. Glycine; 4. Citrate; 5. Trimethylamine N-Oxide; 6. Lactate; 7. Alanine; 8. Dimethylamine; 9. Taurine; 10. Methyl Succinate; 11. Leucine/ Isoleucine; 12. Valine; 13. Phenylacetate; 14. Acetic Acid; 15. Phenylacetylglutamine; 16. Threonine; 17. P.Cresol; 18. Acetoacetate; 19. Succinate; 20. Pyruvate; 21. Isocitrate; 22. Aspartate; 23. Trimethylamine; 24. Tyrosine; 25. Histidine; 26. Glutamate; 27. Betaine; 28. Serine; 29. Guanidoacetate; 30. Methionine; 31. Formate.

The CRC spectrum indicated the presence of specific biological component which does not exist in healthy spectrum. Their intensity depends on the concentration of corresponding metabolites.

Statistical model for the observed spectra

Identification of metabolite variation: The variation of metabolites component from 155 samples is identified by *unsupervised model-PCA* with 4 PCs as in Figure 2:

The confidence level is 95% with 56.68% variances explained by PC1 and 8.17% variances explained by PC2. In Figure 2A, the PC1 vs PC2 scores plot showed ***a trend for separation of 2 groups***, one corresponds to cancer patients and the other to healthy persons, mostly along PC1.

Figure 2. Principal component analysis model (PC1-PC2) of CRC urine and healthy urine specimens. (A) : PCA Score Plot displays the discrimination between healthy samples (circle) and CRC samples (triangle) (B) : 1D-PCA Loading Plot shows the resonances that contribute to the variance on PC1.

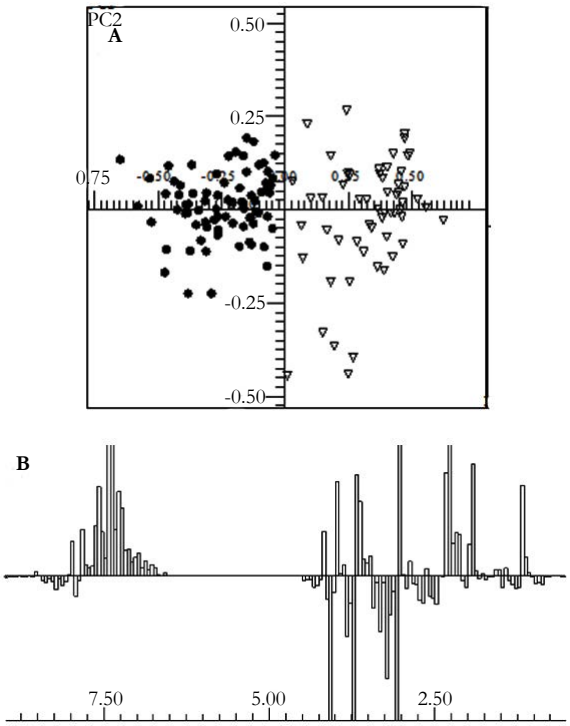


Figure 3. PLS T/T score plot displays the discrimination of healthy samples (circle) and CRC samples (triangle) with the proportion of variance explained by principal component PC1 and PC2.

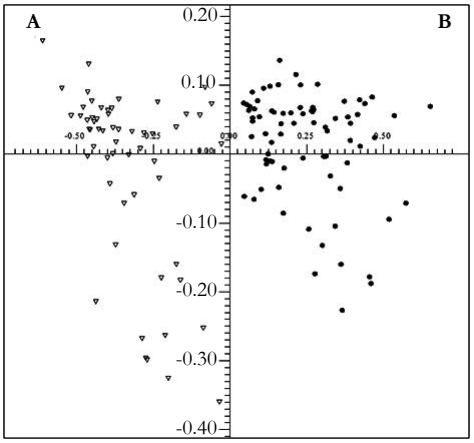


Figure. 2B indicates metabolites which contributed significantly to the separation of 2 groups on PC1. The set of ***marker metabolites*** corresponding to these variables were identified.

We notice strong increase of the peaks corresponding to the following metabolites: hippurate, glycine, valine, phenylacetate, phenylacetylglutamine, p-cresol, succinate, tyrosine. The observed enhancement is mostly much higher than that mentioned earlier by Qiu Y [22]. The presence of proton donors (-OH, -NH) and acceptors (>C=O, >N-) in the enhanced metabolite molecules suggests their possible bonding with ADN nucleotides and involves in the mechanism of carcinogenesis [14].

Some metabolites decreased including creatinine/creatine, histidine, betaine (Table 1). A higher number of samples increases the confidence for their variation.

Prediction of urine-type using Supervised model: Partial Least Squares (PLS)

In addition, we investigated a supervised methods PLS in order to construct a predictive model that is capable of distinguishing between CRC and healthy urines. In the T/T scores of PLS model, a reasonably good separation was observed. The obtained class discrimination was explained X variances up to 71.54% and Y variances up to 87.37%

If the distinction of 2 groups is reconfirmed by supervised model-PLS we have not neglect the possible contribution of other cancers.

Of course, more samples would be examined to reinforce the

Table 1. Relative intensity variation of metabolites.

Peak	Metabolite	Our results	Qiu Y et al. [22]	Young et al. [23]	Doreen et al. [24]	Jin-Lian et al. [25]
1.	Creatinine / creatine	-		-		
2.	hippurate	+ +	+			
3.	glycine	+		-	+	
4.	citrate	-	-	-		-
5.	trimethylamine N-oxide	-		-		
6.	lactate	-				+
7.	alanine	-		-		
8.	dimethylamine	-				
9.	taurine	-		-		
10.	methyl succinate	+	-		+	-
11.	Leucine / isoleucine	-				+
12.	valine	+ +				+
13.	phenylacetate	+ +	+			
14.	acetic acid	+		-		
15.	phenylacetylglutamine	+ +	+			
16.	threonine	-			+	
17.	p.cresol	+ +	+			
18.	acetoacetate	+				
19.	succinate	+ +	-		+	-
20.	pyruvate	-				
21.	isocitrate	-	-	-		
22.	aspartate	-				-
23.	trimethylamine	-		-		
24.	tyrosine	+ +			+	
25.	histidine	-	-	-		-
26.	glutamate	+	+			
27.	betaine	-		-		
28.	serine	-		-		-
29.	guanidoacetate	-				
30.	methionine	-				-

above conclusion in the aim of a better understanding about the disease evolution. Once the metabolomic 'fingerprint' of CRC is firmly established, the next step would be to test the accuracy of this 'fingerprint' and these metabolites in a prospective blinded study against the reference standard by GC-MS or by LC/MS/MS. Success with metabolomics as a diagnostic and prognostic tool is likely to fundamentally change the physicians' approach to human healthcare.

Conclusion

The information obtained from this study proves that the disease state of CRC induced characteristic changes in the urine metabolites. To fully develop this technology, further study would be necessary with a much larger data set. Such a model could be used in human clinical medicine as a non-invasive diagnostic tool, catching more cases of CRC at an early stage, increasing survival rates. Furthermore, some metabolites with strong enhancement variation would intervene in the interaction with ADN nucleotides in the mechanism of carcinogenesis.

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