Metabolic phenotyping of human blood plasma: a powerful biomarker to discriminate between cancer types?

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INTRODUCTION

- The application of metabolomics in the search for cancer biomarkers has increased enormously in the past decade. This discipline comprises the simultaneous and comprehensive analysis of metabolites within a biological system.
- Metabolites constitute the end products of cellular metabolism and therefore changes in their concentration reflect alterations in the metabolic phenotype.
- Accumulating evidence has shown that the metabolism of cancer cells differs from that of normal cells. More specifically, the entire metabolism of cancer cells is reorganized to favor anabolic reactions which induce cell growth and survival. Disturbances in biochemical pathways which occur during the development of cancer provoke changes in the metabolic phenotype.
- Although many studies have already focused on the deregulated metabolism of cancer cells, up to now it is not yet clear whether different cancer types share the same metabolic derangements or whether certain derangements are limited to specific cancer types.
- Nuclear magnetic resonance (NMR) spectroscopy enables a fast, non-invasive identification and quantification of complex mixtures of metabolites, as in blood plasma, in a single run and without extended sample preparation.

OBJECTIVES

1) Investigate whether the metabolic phenotype of blood plasma determined by ^{1}H-NMR spectroscopy allows to discriminate between 80 female breast cancer patients with an adenocarcinoma and 54 female lung cancer patients with an adenocarcinoma (**training cohort**)
2) Examine the predictive accuracy of the metabolic phenotype by external validation in an independent **validation cohort** of 60 female breast cancer patients with an adenocarcinoma and 81 male lung cancer patients with an adenocarcinoma
3) Explain the disturbed biochemical pathways in both cancer types

SUBJECTS AND METHODS

Study population

The lung cancer patients from both cohorts were included in the Limburg Positron Emission Tomography center (Hasselt, Belgium) and at the Department of Respiratory Medicine of University Hospitals Leuven (Leuven, Belgium). The diagnosis of lung cancer was confirmed by means of an pathological biopsy or by a medical doctor with expertise in radiological or clinical data. The breast cancer patients from both cohorts were included at the day of primary surgery at the Department of Gynaecology of Ziekenhuis Oost-Limburg (Genk, Belgium). The diagnosis of breast cancer was confirmed by means of a core needle biopsy. The study was approved by the ethical committees of Ziekenhuis Oost-Limburg (Genk, Belgium), Hasselt University (Hasselt, Belgium) and University Hospitals Leuven (Leuven, Belgium).

^{1}H-NMR spectroscopy

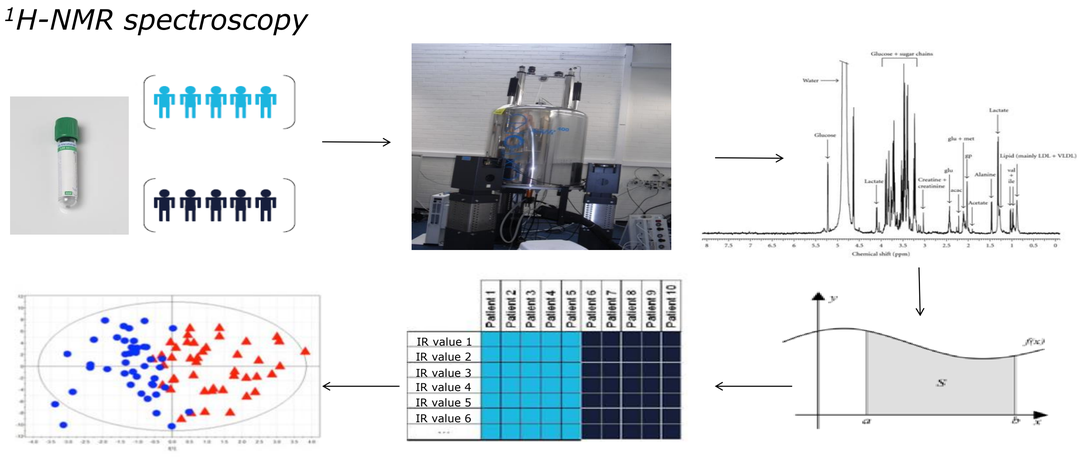

Figure 1. Determination of the metabolic phenotype by ^{1}H-NMR-based metabolomics.

- Collection of fasting venous blood samples from lung cancer and breast cancer patients.
- Analysis of the metabolic composition of blood plasma by a 400 MHz NMR spectrometer.
- The ^{1}H-NMR spectra were segmented into 112 variable-sized spectral regions based on spiking experiments with known metabolites. After excluding water and TSP, the remaining 110 regions were integrated and normalized relatively to the total integrated area of all regions, resulting in 110 normalized integration values, being the variables for multivariate statistics.

Multivariate statistics

- Orthogonal partial least squares discriminant analysis (OPLS-DA) was used to construct a statistical classifier (model) to discriminate between lung cancer and breast cancer patients.
- The predictive accuracy of the model was assessed by means of external validation in an independent cohort. Furthermore, model robustness was evaluated by means of a receiver operating characteristic (ROC) curve.
- To identify the most differential variables, the S-plot and variable importance for the projection (VIP) values of the constructed model were studied in detail. Variables with a VIP value exceeding 0.5 were considered in order to explain the disturbed biochemical pathways in lung and breast cancer.

RESULTS

Table 1. Subject characteristics of the training and validation cohort.

	Training cohort		Validation cohort	
	Breast cancer patients	Lung cancer patients	Breast cancer patients	Lung cancer patients
Number of subjects	80 F	54 F	60 F	81 M
Age	58 ± 11	61 ± 10	60 ± 12	66 ± 9
BMI	26.8 ± 4.9	25.0 ± 5.4	25.9 ± 4.7	25.8 ± 4.1
Smoking habits	-: 55 (69%)	-: 28 (52%)	-: 49 (82%)	-: 44 (54%)
	+: 18 (22%)	+: 26 (48%)	+: 9 (15%)	+: 37 (46%)
	?: 7 (9%)	?: 0 (0%)	?: 2 (3%)	?: 0 (0%)
Menopause	+: 62 (78%)	+: 44 (81%)	+: 41 (68%)	
	-: 18 (22%)	-: 10 (19%)	-: 19 (32%)	
ER status	+: 64 (80%)		+: 51 (85%)	
	-: 16 (20%)		-: 9 (15%)	
PR status	+: 56 (70%)		+: 46 (82%)	
	-: 24 (30%)		- 14 (18%)	
HER 2 status	+: 14 (17%)		+: 11 (18%)	
	-: 66 (83%)		-: 49 (82%)	

Table 2. Stage of the lung and breast tumors in the training and validation cohort.

		Training cohort		Validation cohort	
		Breast cancer patients	Lung cancer patients	Breast cancer patients	Lung cancer patients
Stage	I	29 (36%)	11 (20%)	23 (38%)	16 (20%)
	II	43 (54%)	6 (11%)	33 (55%)	8 (10%)
	III	8 (10%)	17 (32%)	3 (5%)	26 (32%)
	IV	0 (0%)	20 (37%)	1 (2%)	31 (38%)

Discrimination between lung cancer and breast cancer patients based on the metabolic phenotype

- The metabolic phenotype allows to classify 93% of the female lung cancer patients with an adenocarcinoma and 99% of the female breast cancer patients with an adenocarcinoma correctly with an AUC of 0.961.
- Furthermore, it classifies 89% of the male lung cancer patients with an adenocarcinoma and 82% of the female breast cancer patients with an adenocarcinoma from an independent cohort correctly with an AUC of 0.935.

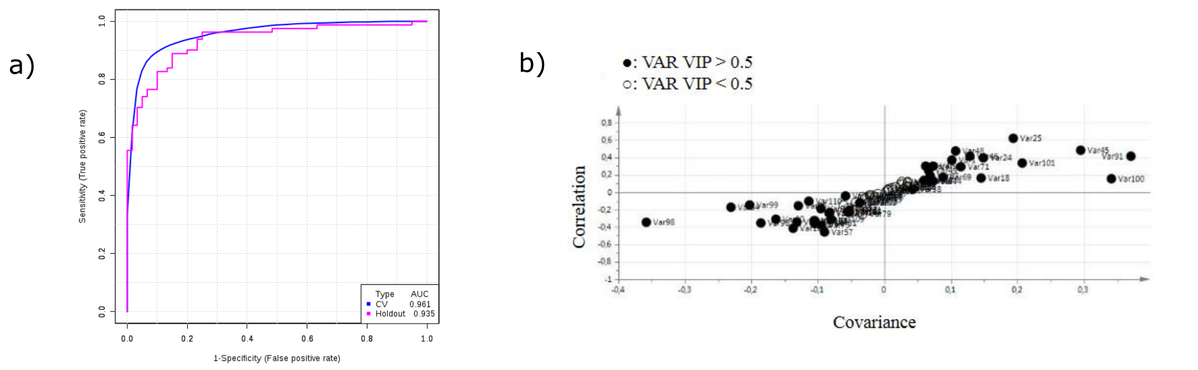

Figure 2. a) ROC curves showing for the cross-validation (CV) as well as for the independent validation (hold-out) a high predictive accuracy of the OPLS-DA model, b) S-plot of the complete OPLS-DA model showing the variables contributing most to the group differentiation. Variables which are used to explain the disturbed biochemical pathways in both cancer types are marked (●).

Unraveling the disturbed metabolism in breast cancer and lung cancer

- The disturbed metabolism in lung cancer as compared to breast cancer is depicted in **Figure 3**. The metabolic changes, which can be linked to the fact that lung tumors are more aggressive and consequently more metabolically active than breast tumors, are reflected by a stronger counteraction of the body in response to the Warburg effect in lung cancer patients. More specifically, increases in hepatic glycogen degradation, ketogenesis and de novo synthesis of glucose to compensate for the lack of glucose for normal cells are more pronounced in lung cancer patients. Consequenty, The Krebs cycle is more hampered in lung cancer patients.

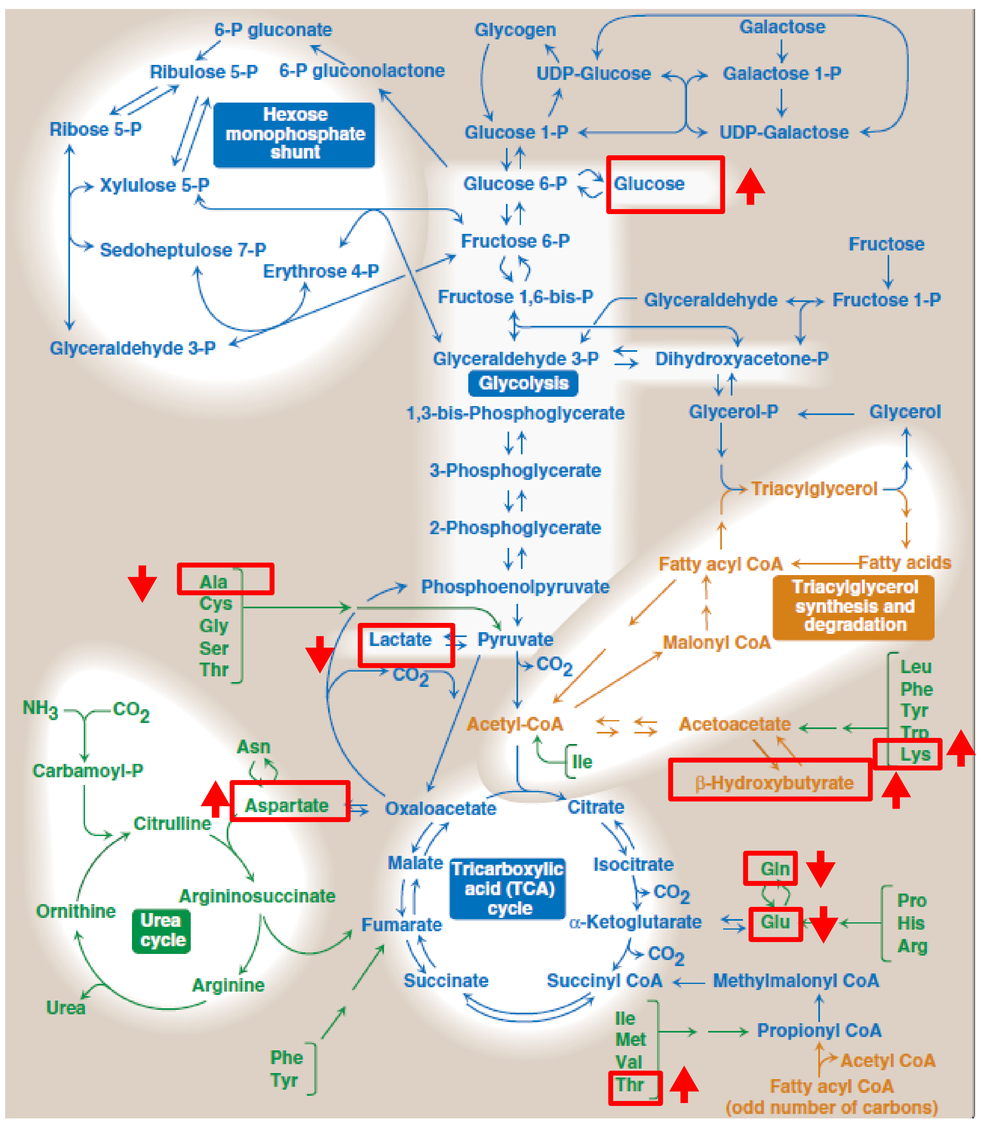

Figure 3. The disturbed biochemical pathways in lung cancer as compared to breast cancer.

CONCLUSION

- Metabolic phenotyping of blood plasma by ^{1}H-NMR spectroscopy seems to discriminate significantly between breast and lung cancer.
- Although our results already give an indication of which metabolites may serve as general cancer biomarkers and which are more specific for either lung or breast cancer, future research is needed (and ongoing) to investigate whether the metabolic phenotype allows to discriminate between other frequently occurring cancer types.

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