Metabolomic assessment of succinate dehydrogenase dysfunction in pheochromocytomas and paragangliomas by 1H-HRMAS NMR spectroscopy: clinical and pathophysiological implications

Alessio Imperiale[1,2], Stéphanie Battini[1], Philippe Roche[3], François-Marie Moussallieh[1], Ercument A Cicek[4], Frédéric Sebag[5], Laurent Brunaud[6], Anne Barlier[7], Karim Elbayed[1], Anderson Loundou[8], Philippe Bachellier[9], Bernard Goichot[10], Constantine A Stratakis[11,12], Karel Pacak[13,14], David Taieb[15], and Izzie-Jacques Namer[1,2]

[1]ICube laboratory UMR 7357, University of Strasbourg/CNRS and FMTS, Strasbourg, France, [2]University Hospitals of Strasbourg, Department of Biophysics and Nuclear Medicine, Hautepierre Hospital, Strasbourg, France, [3]Integrative Structural & Chemical Biology (iSCB) & INT-3D Molecular Modeling Platform, Cancer Resear, CNRS UMR7258; INSERM U1068; Institut Paoli Calmettes; Aix-Marseille University UM105, Marseille, France, [4]Lane Center for Computational Biology, School of Computer Science, Carnegie Mellon University, 5000 Forbes Ave, Pittsburgh, PA 15222, United States, [5]Department of Endocrine Surgery, La Timone University Hospital, Aix-Marseille University, Marseille, France, [6]Department of Digestive, Hepato-Biliary and Endocrine Surgery, Brabois University Hospital, Nancy, France, [7]Laboratory of Biochemistry and Molecular Biology, Conception Hospital, Aix-Marseille, University, Marseille, France, [8]Department of Public Health, Aix-Marseille University, Marseille, France, [9]Department of Visceral Surgery and Transplantation, Hautepierre Hospital, University Hospitals of Strasbourg, Strasbourg, France, [10]Department of Internal Medicine, Diabetes and Metabolic Disorders, Hautepierre Hospital, University Hospitals of Strasbourg, Strasbourg, France, [11]Section on Genetics and Endocrinology (SEGEN), Program on Developmental Endocrinology and Genetics (PDEGEN), Bethesda, United States, [12]Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, United States, [13]Program in Reproductive and Adult Endocrinology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, United States, [14]National Institutes of Health, Bethesda, United States, [15]La Timone University Hospital, European Center for Research in Medical Imaging, Marseille, France

Purpose

Pheochromocytomas/paragangliomas (PHEOs/PGLs) are characterized by high genetic heterogeneity. Mutations in succinate dehydrogenase (SDH) genes (*SDHx*) increase susceptibility to develop PHEOs/PGLs. The *SDHx* genes encode the SDH enzyme that catalyzes the oxidation of succinate to fumarate in the tricarboxylic acid cycle (TCA) and the respiratory chain. The aim of the present study was to investigate the HRMAS NMR-based metabolomic profiling of PHEOs/PGLs in order to: (a) define the global metabolomic profile of the SDH-related PHEOs/PGLs in comparison to sporadic tumors and (b) identify metabolites that could be used as clinical predictors of SDH deficiency.

Methods

Seventy-one specimens of PHEOs/PGLs of sympathetic origin (48 sporadic and 23 *SDHx*-related tumors) were analyzed. No treatment was performed prior to surgery. Tissue specimens were collected after tumor removal and snap-frozen in liquid nitrogen before storage at −80ºC. HRMAS NMR spectra were recorded on a Bruker Advance III 500 spectrometer operating at a proton frequency of 500.13 MHz. For each sample, one 10 min 1D proton spectrum using a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence with water presaturation was acquired for metabolite identification and quantification. Spectra were referenced by setting the lactate doublet chemical shift to 1.33 ppm. HRMAS NMR signals were bucketed into integral regions 0.01 ppm wide (ppm range, 8.65–1) using AMIX 3.8 software and exported into SIMCA P. In order to confirm resonance assignments, 2D heteronuclear (^{1}H-^{13}C) experiments were recorded immediately after the end of 1D spectra acquisition in few cases. A combination of PCA and OPLS-DA was herein adopted. Cross-validation was used to determine the number of components and to avoid data overfitting. The Spearman's non-parametric test was performed to determine the correlation between metabolites. Comparisons of tumor metabolite concentrations between *SDHx*-related and sporadic tumors were performed using a Mann-Whitney U test. The ROC curves were used to evaluate the clinical utility of metabolite quantification in the diagnosis of tumors related to *SDHx* mutation. Linear Regression Model was used to examine the association between metabolites. Network analysis was done by the Algorithm to Determine Expected Metabolite Level Alterations Using Mutual Information (ADEMA).

Results

A two-component OPLS-DA showed a very clear separation between sporadic and *SDHx*-related tumors ($R^2Y = 0.82$, $Q^2 = 0.7$, **Figure 1**). Compared to sporadic, *SDHx*-related PHEOs/PGLs exhibit a specific metabolic signature characterized by increased levels of succinate ($p<0.0001$), methionine ($p=0.002$), glutamine ($p=0.002$) and myo-inositol ($p<0.0007$) and decreased levels of glutamate ($p<0.0007$), regardless of their location and catecholamine levels. Uniquely, ATP/ascorbate/GSH were found to be associated with the secretory phenotype of PHEOs/PGLs, regardless of their genotype ($p<0.0007$). The use of succinate as single screening test retained excellent accuracy in distinguishing *SDHx* vs. non-*SDHx*-related tumors (Se/Sp: 100/100%, cut-off value: 0.096 nmol/mg). ROC curves were also built using the quantitative values of glutamate, methionine, myo-inositol and methionine/glutamate ratio. However, the diagnostic accuracy obtained for each single metabolite was lower than the accuracy achieved by succinate as a single tumor biomarker. When the data is analyzed using the ADEMA algorithm (**Figure 2**), the results showed that network and mutual information based analysis is in accordance with the statistical significance of changes shown above, with the exception of aspartate.

Figure 1

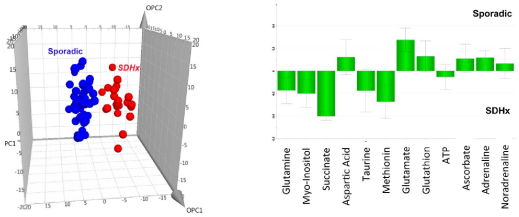
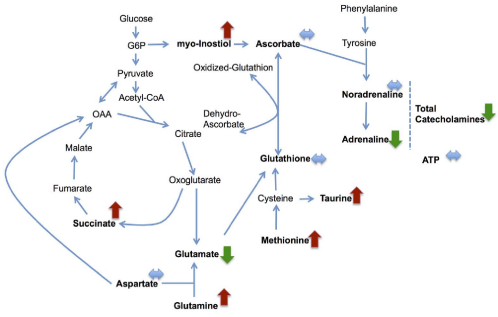


Figure 2



Discussion

The present study shows that HRMAS NMR spectroscopy is a very reliable method for classifying various PHEOs/PGLs according to their genetic background. Such a metabolomics based approach allows for the detection of metabolic changes (biomarkers) that are specifically related to a medical condition (e.g., *SDHx*-related mutation), resulting in diagnostic and potential prognostic implications. In our study, HRMAS NMR spectroscopy allowed for the exploratory investigation of the global metabolic phenotype of *SDHx*-related PHEOs/PGLs, leading to the identification of several specific biochemical alterations. Recent introduction of cryogenic probes has already improved spectral signal-to-noise ratios, reducing the gap between HRMAS NMR and MS. In comparison to 1D ^{1}H-NMR spectroscopy, 2D techniques are further improving both detection sensitivity and metabolite identification, especially when specific metabolite-related picks are overlapped in 1D NMR acquisitions. However, the large centrifugal forces applied to the biological sample during several hours of 2D NMR acquisition, have a direct consequence on tissue integrity, which could potentially influence results.

Conclusion

The present study well justifies that, in the near future, functional genomics will allow for and perfect the identification of tumor-specific metabolic biomarkers as well as their genetics. It is expected that cancer metabolome will be quickly implemented in new diagnostic and treatment options of various cancers as well as their prognosis.