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Abstract

NMR-based metabolomic analysis of biological fluids to monitor relevant unsolved diseases

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Metabolomics and metabonomics encompass the comprehensive profiling of multiple metabolite concentrations and their cellular and systemic fluctuations in response to drugs, diet, lifestyle, environment, stimuli and genetic modulations, in order to characterize the beneficial and adverse effects of such interactions. In the context of biomedical applications, metabolomics will have a preferential role with respect to the other "Omics" sciences for its ability to detect in real time the response of the organisms to pathological stressors. The application of the NMR technique for the metabolomics analysis was applied to bio-fluids deriving from populations of patients respectively affected by salivary gland tumor, antiphospholipid autoimmune syndrome and altered lipid profile. This NMR metabolomic screening was aimed i) at the definition of a metabolomic profile that may be patognomonic of the disease under scrutiny and ii) at the identification of biomarkers to be used with diagnostic and prognostic scope. In the present work, we present a NMR-based metabolomic study of saliva of patients suffering of salivary gland tumors. Our data show that individuals suffering parotid tumor have a characteristic metabolomic profile with abnormalities associated to the metabolism of acetate, alanine, lactate, methanol, phenylalanine, propionate, succinate. We have identified for the first time the metabolomic fingerprint characterizing parotid tumor patients disease having potential application to improve timely diagnosis and appropriate therapeutic approaches. Salivary gland tumor, as many other cancers, is a complex disease, resulting from an interdependent series of biochemical alterations, rather than a single disruptive event. In this case our approach aimed at the identification of a panel of metabolite markers rather than a single biomarker, will improve the sensitivity and specificity for detection. Integrating the protocols of tumor grading and histological classification. Our NMR-based metabolomic study revealed different metabolomic profiles in saliva of male patients affected by salivary gland tumors compared with the profiles of age, gender, and sampling-date matched control individuals. Our approach provide preliminary data for the identification of metabolites that can be used as metabolomics fingerprint of salivary gland tumor. Determination of metabolomics fingerprint, rather than

single metabolic biomarker, may fully reflect the multifactorial nature of oncogenesis and the heterogeneity of oncogenic pathways, providing precious elements to integrate diagnostic laboratory and clinical tests. Antiphospholipid syndrome (APS) is a rheumatic inflammatory chronic autoimmune disease inducing hypercoagulable state associated with vascular thrombosis and pregnancy loss in women. Cardiac, cerebral and vascular strokes in these patients are responsible for reduction in life expectancy. Timely diagnosis and accurate monitoring of disease is decisive to improve the accuracy of therapy. In the present work, we present a NMR-based metabolomic study of blood sera of APS patients. Our data show that individuals suffering APS have a characteristic metabolomic profile with abnormalities associated to the metabolism of methyl group donors, ketone bodies and amino acids. We have identified for the first time the metabolomic fingerprint characterizing APS disease having potential application to improve APS timely diagnosis and appropriate therapeutic approaches. The first stratification of APS patients according to the gender offers preliminary indications for the management of the disease according to the gender oriented medicinal approach. Human serum includes a large number of components which derive from endogenous metabolism and nutritional intake. Serum components vary in response to diet. Serum lipid composition is probably the most important benchmark in assessing cardiovascular risk and disease progression. Serum components, also derived from nutritional intake, can affect general metabolism and, more specifically, affect molecular mechanisms and pathways linking nutritional intake and chronic disease risk. To identify the effect exerted by altered lipid composition on the genome expression pattern, response of gene expression to serum samples from hypercholesterolemic and normocholesterolemic male subjects was previously studied. In the present part of my PhD thesis, using a NMR metabolomics approach I studied the metabolomics profile of the aforementioned hypercholesterolemic and normocholesterolemic sera to correlate the previously identified trascriptomic signature of human hepatoma cells to the relative metabolomics profile. Hypercholesterolemic sera previously proved to increase in human hepatoma cells, the mRNA expression of HMGCS2, an enzyme involved in the pathway of keton bodies. Our NMR based metabolomics analysis evidences abnormal concentrations of metabolites involved in the keton bodies pathway. This indicates a correlation between the trascriptomic profile of hepatoma cells treated with hypercholesterolemic sera, and the metabolomics profile of the same sera.