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MOLECULAR STUDY OF PORTUGUESE PATIENTS WITH CLINICAL DIAGNOSIS OF SHWACHMAN-DIAMOND SYNDROME

E. Costa,[1] J. Oliveira,[2] E. Vieira,[2] F. Duque,[3] P. Garcia,[3] I. Gonçalves,[3] L. Diogo,[3] J. Barbot,[4] R. Santos[2]

[1]Instituto Politécnico de Bragança, BRAGANÇA; [2]Unidade de Genética Molecular, IGM, PORTO; [3]Hospital Pediátrico de Coimbra, COIMBRA; [4]Hospital de Crianças Maria Pia, PORTO, Portugal

Shwachman-Diamond syndrome (SDS; MIM# 260400) is a rare autosomal recessive disorder characterized by the association of exocrine pancreatic and bone marrow dysfunction. Other systemic findings (skeletal, liver and psychomotor) or problems secondary to bone marrow dysfunction may also be detected. Intermittent or persistent neutropenia is the most common haematological finding, but anaemia and thrombocytopenia are present in approximately 40% of the patients. The SDS locus was mapped to chromosome 7q11 where disease-associated mutations were later reported in the Shwachman-Bodian-Diamond syndrome gene (SBDS; MIM# 607444). The present report summarises the molecular analysis of 14 Portuguese patients with suspected diagnosis of SDS. Direct sequencing of all coding regions of the SBDS gene, including exon-intron boundaries, was conducted in all cases. Additional molecular studies including long-range PCR and cDNA analysis were performed to clarify the involvement of a refractory mutation in a case where only a heterozygous mutation had been detected by direct sequencing. Four patients were found to be compound heterozygous for the common c.181_184TA>CT and c.258+2T>C mutations. One of the patients was a compound heterozygote for the c.258+2T>C mutation and a previously unreported genomic deletion (c.258+374_459+250del). This novel mutation, predictably giving rise to an internally deleted polypeptide (p.Ile87_Gln153del), appears to have arisen from an excision event mediated by AluSx elements present in introns 2 and 3 of SBDS gene. In the remaining nine patients no pathogenic mutations were found. Eight previously reported polymorphisms were also detected in the course of this study (c.129-162TTGGGGGTAAGAAAdelinsGGGGGCGGGGG, c.129-71G>A, c.141C>T, c.201A>G, c.258+54T>G, c.459+92A>G, c.635T>G and c.651C>T). The previously reported mutations c.181_184TA>CT and c.258+2T>C, arising from gene conversion events, are the most frequent mutations associated with SDS in this group of patients. The detection of a large genomic deletion encompassing exon 3 illustrates the importance of screening for gross rearrangements, especially in patients where a single mutation is detected by routine methods. The considerable number of patients in which no mutations were found, may be explained by variations in intronic or regulatory regions that were not screened, the involvement of other genes acting in a common pathway, or broad selection criteria permitting inclusion of misdiagnosed patients.

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METHYLATION PROFILE IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA

D. Marinitch,[1] N.D. Volcovets,[1] D.G. Tsvirko,[1] Y.L. Buryanov,[2] T.V. Shevtchuk,[2] O.V. Dyachenko[2]

[1]Byelorussian Hematology Center, MINSK, Rebublic of Belarus; [2]Institute of Bioorganic Chemistry, PUSHTCHINO, Russian Federation

Epigenetic (nonmutational) changes in the promoter region of multiple genes are very important in leukemia triggering and development including chronic myeloid leukemia (CML). To further characterize this event, the methylation status of the 5'-promoter region of multidrug-resistance (MDR-1) gene and calcitonin (CT) gene in CML patients was investigated. Totally 26 DNA samples obtained from mononuclear cells of peripheral blood and bone marrow of 18 CML patients were investigated. Ten patients were in chronic phase of the disease, 5 - in accelerated stage and 3 - in blast crisis. As a control we used genomic DNA obtained from 10 healthy donors. We applied methyl-dependent polymerase chain reaction (PCR) method, investigating the intensity of CpG-sites methylation of both genes in the 5'-promoter area. The methylsensitive (HpaII) and methyl insensitive (MspI) restriction endonucleases were used for the digestion of DNA-samples. The products of the PCR were scanned directly in agarose gel. According to the data of the experiments, the intensity of the MDR-1 gene methylation progressively decreased from stage to stage of CML. Conversely, the methylation level of 5'-CT-gene became higher. The CT/MDR-1 optical density ratio was used for the semiquantitative analysis. We revealed a sustained increase of this index during the disease progression. The mean donor (normal volunteers) index was 0.44±0.14, whereas the similar figures in CML were 0.82±0.21 in chronic phase; 1.35±0.17 in accelerated phase and 2.19±0.4 in blast crisis. It is interesting to notice, that this index was higher in patients (in chronic phase), who received imatinib, compare to those, who was under conventional (hydrea) therapy. It is well known, that DNA-methylation often reversely correlates with gene expression. Thus, the revealed hypomethylation state of 5'-region of the MDR-1 gene suggests that the progression of CML is likely accompanied by acquisition of multidrug-resistance phenotype. CT-gene hypermethylation, as it was stated earlier, strongly correlates with the loss of expression of tumor suppressor genes. Both components are crucial in the illness progression. These findings might be useful in the disease monitoring and also serve as a marker of treatment efficacy in CML.

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IDENTIFICATION OF NEW PROTEINS INVOLVED IN THE PATHOGENESIS OF THE ANTIPHOSPHOLIPID SYNDROME BY PROTEOMIC ANALYSIS: EFFECTS OF *IN VIVO* STATINS TREATMENT

C. Lopez-Pedrera,[1] M.J. Cuadrado,[2] V.H. Hernandez,[1] M.A. Khamashta,[2] P. Buendia,[1] M.A. Aguirre,[1] N. Barbarroja,[1] L.A. Torres,[1] F. Velasco[1]

[1]Reina Sofia Hospital, CORDOBA, Spain; [2]St Thomas Hospital, LONDON, United Kingdom

Background. Antiphospholipid syndrome (APS) is an autoimmune disease manifested by thrombotic or obstetrical events in the presence of antiphospholipid antibodies (aPL). In spite of the recent progresses, many aspects of this disease remain unclear, such as the molecular mechanisms leading to thrombosis. In addition to its anti-inflammatory and immunomodulatory properties, statins have been shown antithrombotic effects, although the molecular mechanisms leading to this effect are not yet fully understood. This study analyzed, by using proteomic techniques: a) changes in proteins expression of monocytes from APS patients related to the pathophysiology of the syndrome; and b) the *in vivo* effects of Fluvastatin on the pattern of protein expression in this cellular setting. *Patients and Methods.* Proteomic analyses were performed in 25 APS patients. Control groups included: 10 patients with aPL antibodies but without previous thrombosis, 10 patients with previous thrombosis but without aPL antibodies, and 10 healthy donors. Ten patients with APS and previous history of thrombosis further received Fluvastatin (20 mg/day) for one month. Blood samples were obtained before treatment and after one and three months of treatment. All patients were tested for the presence of anti-cardiolipin autoantibodies and lupus anticoagulant. Monocytes were isolated from peripheral blood mononuclear cells by magnetic depletion of non-monocytes. Proteomic analyses were performed using two-dimensional electrophoresis and MALDI-TOF mass fingerprinting analysis. *Results.* Approximately 500 protein spots were detected on the comassie-stained gels from the donors' material. Proteins identified as more significantly altered between monocytes from APS patients and controls' donors belonged to the group of signal transduction mediators (i.e. lipocortin I, Annexin II, Rho A proteins, Ubiquitin conjugating enzyme, zinc finger proteins), metabolic enzymes (i.e. α-enolase, ATP-synthase) and immunomudulators (i.e. Hsp60, disulfide isomerase). Some of these differentially expressed proteins (such as annexin II, Rho A proteins and Hsp60) have previously been shown to play a relevant role in the pathogenesis of the APS. *in vivo* statins treatment for one month reversed the changes observed in the expression levels of those proteins. These levels then suffered a slowly return, although remained significantly changed in relation to control values after three months of the end of the treatment. To confirm the results, more specific analytical techniques, such as real time RT-PCR and Western blot were used. Moreover, to assess the effect of aPL on monocyte proteomics, monocytes from normal individuals were treated with affinity purified patients' IgG, in the presence or in the absence of fluvastatin. All those studies further supported the data presented. *Conclusions.* Our study has identified, by proteomic analysis, for the first time: 1) some proteins that may be involved in the pathogenic mechanisms of the APS syndrome; and 2) the changes that protein patterns of monocytes from APS patients suffered when statins treatment was used for 1 month. These findings might provide new targets for rational pathogenesis-based therapies of APS.

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