

Pyruvate Kinase Deficiency in *Escherichia coli*, Human Erythrocytes and Mice: Genomic and Proteomic Application to Study Metabolic Regulation.

Prabhakar Shivramji KEDAR

DST - 10435

Technical Officer,
Indian Council of Medical Research, Institute of Immuno haematology

Japanese Advisor : Kazuyuki SHIMIZU
Professor, Kyushu Institute of Technology

Effect of *pyk-F* gene knockout on the metabolism in *Escherichia coli* based on genomic (DNA Microarray) and proteomic application (2DE and MALDI-TOF- MS): The proteomic response was quantitatively analyzed using one dimensional (1DE) and two-dimensional gel electrophoresis (2DE) followed by identification with matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS). Protein expressions of the mutant strain were compared with the wild type *E. coli*, where 24 protein spots were expressed differentially. Ten of these, which were remarkably increased in the mutant *E. coli*, were identified by MALDI-TOF-MS analysis. Evidently, main metabolic enzymes from the aromatic amino acid biosynthetic pathway such as DAHP synthase (*aro F, G, H*), shikimate dehydrogenase (*aroE*), shikimate kinase (*aroK, L*), chorismate synthase (*aroC*), prephenate dehydrogenase (*pheA*), aminotransferase, anthranilate synthetase and tryptophane synthetase showed significant up-regulation in the mutant. These results show that the protein expression data were almost consistent with the data obtained from the DNA Microarray as well as enzyme activity. Accumulation of PEP and E4P may direct the metabolic flow towards the biosynthetic route of chorismate and shikimate, key metabolic precursors of aromatic amino acids.

Over expression of these enzymes plays an important role in increasing the production of phenylalanine, tyrosine and tryptophan in the *pykF* mutant.

Molecular characterization of the PK-LR gene in pyruvate kinase deficient Indian patients: Eighteen unrelated PK defi-



cient Indian patients were identified in the last 4 years. The clinical phenotypes varied from a mild presentation in some patients to a severe transfusion dependent disorder in others. We identified seventeen different mutations in the PK-LR gene among the 36 mutated alleles. Eleven novel mutants (427 G→A, 499 C→A, 1072 G→A, 1180 G→T, 1190 A→T, 1216 G→A, 1220 A→G, del644G, Ivs5 (+20) c→a, Ivs9 (+44) c→t, Ivs9 (+93) a→c found in RPK-deficient patients were structurally characterized. Other five-missense mutations (958G→A, 1190A→T, 1219G→A, 1220A→G, 1436G→A and 1456C→T), one three-nucleotide deletion (1042 to1044) was also found in our patient. A severe syndrome was commonly associated with some missense mutations (in particular 992G, 1436A and 1220G), one frameshift mutation, (644G del) and IVS9 (+93) a→c in the PKLR gene. The most frequent mutations in the Indian population appear to be 1436A (19.44%) followed by 1456T (16.66%) and 992G (16.66%). To correlate genotype with the phenotype, mutation was related to the biochemical properties of the mutant enzyme and clinical course of the disease. This is the first comprehensive report on molecular characterization of PK deficiency from India where 11 novel mutations were identified.

A proteomic analysis of CBA-*Pk-I^{slc}*/*Pk-I^{slc}* mice with a homozygous point mutation of the gene encoding Red Blood Cell Type Pyruvate Kinase using 2DE together with MALDI-TOF-MS analysis: We have previously established SLC3, a cell line of Friend erythroleukemic cells from the *Pk-I^{slc}* mouse, which has chronic hemolytic anemia with marked splenomegaly due to a missense mutation of the murine PKLR gene. SLC3 as well as primary erythroblasts showed spontaneous apoptosis. Thus it was suggested that the metabolic disturbance in PK deficiency affects not only the survival of red cells but also the maturation of erythroid progenitors, resulting in ineffective erythropoiesis. Recently, it was also showed that glycolytic inhibition by PKLR gene mutation augmented oxidative stress, leading to the activation of HIF-1 as well as downstream pro-apoptotic gene expression. In this study, the proteomic response of SLC3 was quantitatively analyzed using one dimensional (1DE) and two-dimensional gel electrophoresis (2DE) followed by identification with matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS). We detected significant differences between SLC3 and a control Friend cell, CBA2, where nearly 100 protein spots were found to be expressed differentially. Approximately 72% of the total proteins studied were significantly down regulated in SLC3, where as only 28% of proteins were up regulated. Several RBC membrane proteins such as protein 4.1R, gamma adducing and aquaporin are significantly down regulated in SLC3. Reduction of these membrane proteins may cause reduced stability and integrity of PK deficient RBC. Proteins related to oxidative stress in SLC3 mice were up regulated and antioxidant proteins such as peroxiredoxin 6; glutaredoxin 1 and catalase were down regulated. These proteins may be related to the generation

of reactive oxygen species (ROS). For the refolding of mutant protein, molecular chaperon is expectedly unregulated in SLC3. Up-regulation of two chaperons, HSP90B1 and DSIA3 are presumably an adapted reaction in SLC3. HSPA9A (GRP75, mortalin) is a member of heat-shock protein (HSP70), and quite recently mortalin is reported as a novel mediator of erythropoietin signaling. We speculated that down-regulation of mortalin might be attributable to disturbance of EPO signaling, resulting in premature death of PK-deficient erythroid cells.

