Identification, quantification and characterization of some of erythrocyte membrane proteins in Sri Lankan Polycythemia Vera patients using Mass spectrometry based proteomics

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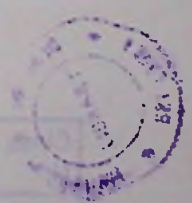

Abstract

Analysis of erythrocyte membrane associated proteins (ERMBPs) of Polycythemia Vera (PV) and healthy individuals in Sri Lanka are presented in this thesis. The main aim of this research is identification and precise quantification of ERMBPs of PV patients and healthy controls employing mass spectrometry based proteomics to understand better the molecular mechanism that underline the PV disease via the information provided by identified ERMBPs.

Blood samples were obtained from five PV patients with the *JAK2 V617F* mutation and five healthy volunteers. All 5 patients were on hydroxycarbamide and low dose aspirin for over 6 months with stable blood counts and had also been subjected to multiple therapeutic venesections by the time of collection. All the fresh blood samples were kept at +4 ^{0}C for 3 days in order to mature reticulocytes to erythrocytes. ERMBPs were isolated by osmotic lysis, followed by several centrifugation and washing steps. The isolated membranes were solubilized by 4% Triton X-100 (in 10mM Tris-HCl, pH 8.8). Then the protein concentration was measured, equal amount of proteins were loaded on to the polyacrylamide gel, subjected to electrophoresis, and silver staining was used to visualize the separated proteins. Gel bands 40-55 KDa were dissected out from gels, digested by trypsin, resulting peptides were extracted, the supernatant containing tryptic peptides was dried by vacuum centrifugation, were dissolved in 2% formic acid, and the solution was analyzed by Liquid Chromatography Mass Spectrometry (LC-MS/MS), QStarElite. The experimentally generated singly charged ions of peptide mass fragments were compared with the theoretical mass values obtained in-silico digestion and identification was made using Mascot database. Quantification of proteins was performed by spectral counting, and quantification of proteins based on precursor ion intensities. The outcomes were validated by targeted quantitative proteomic technique, Selected Reaction Monitoring (SRM).

4 ERMBPs, PDIA1, PDIA6, TXND5 and ERp44, showed statistically significant, 1.5 – 3.5 fold increase in protein abundance in PV patients compared to healthy controls. Characterization of PDIA1, PDIA6, TXND5 and ERPp4 was performed using databases and software. The thioredoxin domains (Trxs) were located by sequence alignments, which were carried out using BioEdit Sequence Alignment Editor and comparing with the structures obtained from PDB. The ribbon cartoons for PDIA1, PDIA6, TXND5 and ERp44 were generated using PyMOL. The Trxs and CGHC motifs were visualized. All 4 proteins belong to the same family called protein disulphide isomerase (PDI). They consist of at least one Trx, which is known to cause formation, breakage and rearrangement of disulphide bonds in proteins related to oxidation stress.

In view of the above findings, as erythrocytes are constantly exposed to oxidative stress, therefore it is hypothesized that increased abundance of 4 PDI proteins containing Trxs could be considered as an inhibiting factor of stress induced apoptosis in erythrocytes and therefore contribute towards the increased erythrocyte mass in PV patients. Hence thioredoxin domain containing PDIAI, PDIA6, TXND5 and ERp44 proteins in PV proteins could be identified as possible targeted by drugs to treat PV disease.