CHANGES IN THE MEMBRANE PROTEOME OF HUMAN CHRONIC KIDNEY DISEASED ERYTHROCYTES

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Anemia of chronic kidney disease (CKD) is attributed to a decreased production of erythrocytes due to a deficiency in erythropoietin (EPO). Administration of EPO is used to reduce anemia in these patients. However, CKD erythrocytes show higher rigidity and reduced half-life, which also contributes to anemia [1]. Factors influencing survival of diseased erythrocytes have been investigated but final conclusions cannot be stated. We hypothesize that the membrane structure may play a role in their flexibility and we investigate possible differences in their membrane proteome by DIGE technology.

Three groups were investigated (four individuals per group): control, nonEPO-treated CKD stage V and EPO-treated CKD patients (dialyzed). Erythrocytes were isolated from plasma, washed three times and lysed in a total of five cycles to isolate the membrane fraction. Membrane proteins were solubilized in 7M urea, 2M thiourea, 4% CHAPS and purified on chromatographic minicolumns prior to CyDye DIGE fluor saturation labelling [2].

The DIGE analysis resulted in a total of 760 detected spots, on average, and 420 spots matched throughout all gels. 33 spots were differentially expressed ($p<0,05$) between at least two of the three groups investigated. PCA analysis confirmed adequate grouping of the samples and clearly differentiates nonEPO-treated CKD patients from the other two groups. Nine differential spots could be identified corresponding to beta-adducin, HSP71/72 and tropomodulin-1. Ezrin, radixin and moesin were also identified but based on peptide sequences which were identical for the three proteins. So, it was not possible to unequivocally attribute significant differences to any of them by mass spectrometry. By WB analysis, we confirmed ezrin and radixin as overexpressed in EPO-treated CKD patients, while moesin could not be detected.

[1] Docci D, et al. Clin Nephrol 23:68, 1985.
[2] Alvarez-Llamas G, et al. Electrophoresis 30:4095, 2009.