Erythrocyte membrane skeleton is a structure formed by peripheral membrane proteins. The erythrocyte membrane skeleton is a very important structural and functional element as this cell does not contain any other skeletal structures. Simultaneously, it experiences a great mechanical stress during circulation and undergoes multiple forced shape changes passing through capillaries diameter of which is often fourfold smaller than the larger dimension of the red cell. For the mechanical properties of the erythrocyte membrane membrane skeleton formed in a result of multiple protein-protein interactions is responsible. Characterization of these interactions help to explain molecular mechanisms of reasonable number of cases of hemolytic anemias, in particular **hereditary spherocytosis**, which occurs mostly families of Caucasian population.
In this disease red blood cells are the subject of hemolysis and first of all due to so called spleen conditioning their removal from circulation by spleen macrophages is markedly accelerated (their life-span is decreased from normal 120 days to 3-4 days). This decrease in erythrocyte count is compensated by increase erythropoiesis, which is usually seen by increased reticulocyte count.
Defects in these interactions due to the lack, decreased level or structure change in one of the protein component of the membrane lead to the loss of red cell surface area and to the spheroidal shape of the erythrocyte in particular loss of the membrane elasticity and mechanical stability are known to be the cause of HS. Despite a great progress noted in the field of erythrocyte membrane skeleton and molecular pathology of erythrocyte there are still many cases of HS and mutations in the genes coding for erythrocyte membrane proteins, in the cases of which molecular mechanisms underlying disturbance of the structure and function of the erythrocyte membrane remain unknown. Learning of these mechanisms should bring an increase of knowledge concerning the role of individual proteins and also their domains in the formation and function of the membrane skeleton and therefore the membrane. Therefore the aim of the proposed project is:
1. **Learning of the details of the molecular mechanism(s) of several cases of hereditary spherocytosis,** known from cases found and described by our team or known from the literature, but difficult to explain by known interaction between membrane skeleton and integral proteins. In the project we plan to resolve several HS cases. Basic study method will be cloning of recombinant domain of erythrocyte membrane proteins and introducing mutation mimicking naturally occurring mutation in the patient erythrocyte. Next the interactions in vitro and in vivo will be characterized by using several methods.
2. **An attempt to identify new, yet undiscovered and not reported in the literature and databases** mutations in genes encoding proteins of the erythrocyte membrane in HS patients of 4 families in which the molecular defect has not been determined yet. In order to achieve this we plan to carry out the exome sequencing of genomic DNA isolated from blood of the HS patients registered and diagnosed in the Clinic of Hematology and Neoplastic Diseases and Bone Marrow Transplantation, Wrocław Medical University or healthy individuals serving as control group. Mutations and polymorphisms will be validated via systematic sequencing of amplified via regular PCR technique of DNA fragments of studied genes. In more complicated cases, when the polymorphism(s) related to the defect would not be possible to resolve we will apply RNA analyses, so called RNAseq. Obtained via this method results will be verified by using real-time PCR. Expected outcome of this project should be detailed characteristics of the molecular mechanism(s) underlying HS connected to the mutations in the tandem ZZUD domain of ANK1 gene encoding erythrocyte ankyrin and also in SPTB gene encoding N- and C-terminal domains of this protein. We anticipate that understanding of these fragments role in membrane skeleton formation should enrich our knowledge concerning pathways of normal and pathological membrane function. We hope that this knowledge enforces physicians in actions improving HS patients quality of life. We expect to enrich human erythrocyte protein encoding genes mutation catalogue what should facilitate full molecular diagnostics of the HS cases.