In the cells there is a constant struggle to maintain the balance between the processes that promote survival and those that lead to cell death. This balance is crucial for the proper functioning of tissues and organism. One of the factors affecting these processes is the oxidative stress. Oxidative stress accompanies all organisms throughout their lives, being intensified by the factors such as ultraviolet radiation, toxins, pathogens. Oxidative stress results from the increased production of reactive oxygen species, which normally remains under the cellular antioxidant and repair systems. Increased oxidative stress contributes to the damage of cell components that can be particularly dangerous for the genetic material. Oxidative damage accumulated over the time leads to the development of serious pathologies. The protein that I am particularly interested in, p66Shc, is an essential element of cellular signalling pathways associated with the initiation of oxidative stress, cell response to oxidative stress and activation of the programmed cell death, thus being involved in maintaining the balance described above. In response to adverse environmental conditions, this protein undergoes modifications, which lead to an increase of reactive oxygen species production. For this reason, the p66Shc participation in the development of many diseases associated with oxidative stress is intensively studied. It has been shown that the lack of the p66Shc protein correlates with prolonged life, but its high level and activation is observed for example in the case of kidney damage caused by hyperglycemia, damage of hepatocytes caused ethanol, and in the toxicity of chemoterapeutics. The p66Shc protein a "two-faced" molecule. It participates not only in the cellular response to oxidative stress (proapoptotic function), but also in the signal transduction (adaptor function). p66Shc function is regulated by its posttranslational modifications and according to the literature data, seems to be related to its localization within the cell. The majority of the protein is located in the cytoplasm and the endoplasmic reticulum, where it plays an adaptor role. Doubts concerning p66Shc are related its proapoptotic function. Initially it was thought that the pool of protein, associated with the production of reactive oxygen species locates in the mitochondrial matrix. Afterwards these data were verified showing that p66Shc interacts with cytochrome c, which suggests its mitochondrial intermembrane space localization. The results of our experiments suggest that p66Shc is located within the mitochondria associated membrane fraction. The mitochondria associated membrane fraction is a compartment consisting of cellular membranes structurally and functionally associated with the outer mitochondrial membrane forming. MAM fraction creates a unique environment for certain processes and proteins. We have shown that the p66Shc protein level is higher in the MAM fraction of old animals than in the young ones. We have found it to be correlated with an increased mitochondrial reactive oxygen species production in aged animals. Thus we suggest that the MAM location of p66Shc determinates its involvement in oxidative stress, and that its interactions within MAM are crucial for the proapoptotic function. For these reasons our aim is to investigate the p66Shc interacting proteins in the mitochondria associated fraction. The research plan consists of three main steps. In the first we will identify proteins interacting with p66Shc by the mass spectroscopy analysis of the proteins co-immunoprecipitated with the use of specific anti-p66Shc antibodies. The second step is aimed on the verification of the interactions using techniques like 2-dimensional electrophoresis (Blue Native electrophoresis followed by SDS-PAGE), co-immunoprecipitation with the antibodies against the proteins potentially interacting with p66Shc, co-localization and FRET (Fluorescence Resonance Energy Transfer) analysis. Finally we will investigate the effect of the p66Shc interacting proteins level and activity modulation on the production of reactive oxygen species and activation of apoptosis. The results will help to understand the p66Shc involvement in the oxidative stress associated pathologies. It will also expand the general knowledge about the participation of p66Shc protein in the signalling pathways associated with the cellular response to oxidative stress. In the future, our findings can help to create strategies preventing the harmful effect of the oxidative stress.