

An attempt to identify proteins interacting with p66Shc in the mitochondria associated membranes.

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An adaptor protein p66Shc, participates not only in mitogenic signalling, but also affects cellular redox state and promotes cell death by increasing mitochondrial reactive oxygen species (ROS) production. Localization of p66Shc in the region of endoplasmic reticulum (ER) closely interacting with mitochondria (mitochondria associated membranes (MAM) fraction), has been previously suggested however the details were still under debate. The aim of the presented study is to identify proteins interacting with p66Shc in MAM in order to clarify the mechanism by which p66Shc influences cellular redox state. To investigate p66Shc interactions with other proteins under physiological conditions, protein complexes were co-immunoprecipitated (co-IP) from MAM fraction samples prepared from mice livers, with the use of five, various, specific, anti-SHCA antibodies and then subjected to mass spectrometry (MS) analysis. The MS analysis revealed 197 potentially interacting proteins among which over 30 are involved in the response to oxidative stress or related to mitochondrial bioenergetics. In our studies we were interested in the group of proteins of mitochondrial or ER origin found in MAM simultaneously related to oxidative stress and mitochondrial bioenergetics. Then we verified whether these selected proteins were present together with p66Shc in the protein complexes isolated from MAM using techniques like two dimensional gel electrophoresis and co-IP. None of the respiratory chain related proteins were confirmed to interact with p66Shc so far. Interestingly, such approach didn't confirm also the interaction of p66Shc with cytochrome c, what was previously proposed in order to explain the involvement of p66Shc in mitochondrial ROS production. Moreover, our data supported the reports showing that p66Shc is not detectable in pure mitochondrial fraction, but its presence has been confirmed in MAM, where it may interact with proteins annotated to other organelles.