ABSTRACT

Mitochondria are multifunctional organelles playing a key role in energy metabolism and cell death induction. Mitochondria, and specifically their respiratory chain, are also the main producers of reactive oxygen species (ROS) in cells. Metabolism can be affected by the state of cellular proliferation and certain ROS–inducing agents have an antiangiogenic effect based on the preferential elimination of proliferating endothelial cells (EC). Therefore, in this work we investigated, whether mitochondria could be responsible for different sensitivity of proliferation and confluent EC to cell death. We mainly focused on systems that regulate ROS level and apoptosis: respiratory chain (ROS production), antioxidant defense (ROS detoxification) and Bcl–2 family of proteins (apoptosis regulation). First, we treated EC with functional and nonfunctional respiratory chain with various oxidative stress– and apoptosis–inducing agents and determined ROS production and susceptibility to apoptosis in proliferating and confluent cells. Our results show that functional respiratory chain greatly increases the susceptibility of proliferating cells to ROS induction and apoptosis, whereas in qiescent cells it protects against cell death. Given these findings, we assessed the activity of respiratory chain in proliferating and quiescent EC and we found that proliferating cells consume more oxygen especially when respiring on complex I substrates. Expression of protein subunits of respiratory chain did not explained this observation. On the other hand, blue native electrophoresis showed that the supramolecular organisation of respiratory complexes to the so called supercomplexes is different in proliferating and confluent EC with the greatest differences for complex III. These results were confirmed by in–gel activity assay of respiratory supercomplexes. We also focused on the role of main antioxidant systems like superoxid dismutase and reduced glutathione in proliferating and confluent cells with functional and defective respiratory chain. However, obtained data suggest that these systems do not decisively regulate ROS levels and apoptosis in these settings. Determination of Bcl–2 family proteins showed that these proteins are expressed similarly in proliferating and quiescent cells irrespective of the respiratory chain functionality, so they are probably not responsible for differences in apoptosis susceptibility either. In conclusion, the increased susceptibility of proliferating EC to oxidative stress and apoptosis is to a large extent determined by the state of the respiratory chain state and thus, mitochondria play the decisive role in this phenomenon. (In Czech).

Key words: mitochondria, oxidative stress, apoptosis, cell respiration, respiratory chain, proliferation