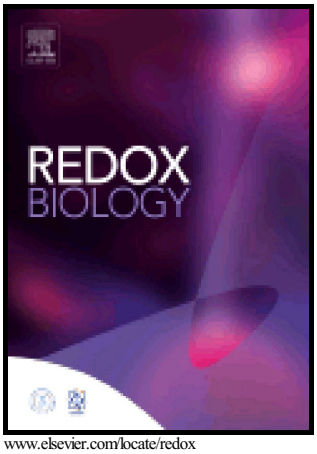

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Redox Environment in Stem and Differentiated Cells: A Quantitative Approach

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Abstract

Stem cells are believed to maintain a specific intracellular redox status through a combination of enhanced removal capacity and limited production of ROS. In the present study, we challenge this assumption by developing a quantitative approach for the analysis of the pro- and antioxidant ability of human embryonic stem cells in comparison with their differentiated descendants, as well as adult stem and non-stem cells. Our measurements showed that embryonic stem cells are characterized by low ROS level, low rate of extracellular hydrogen peroxide removal and low threshold for peroxide-induced cytotoxicity. However, biochemical normalization of these parameters to cell volume/protein leads to matching of normalized values in stem and differentiated cells and shows that tested in the present study cells (human embryonic stem cells and their fibroblast-like progenies, adult mesenchymal stem cells, lymphocytes, HeLa) maintain similar intracellular redox status. Based on these observations, we propose to use ROS concentration averaged over the cell volume instead of ROS level as a measure of intracellular redox balance. We show that attempts to use ROS level for comparative analysis of redox status of morphologically different cells could lead to false conclusions. Methods for the assessment of ROS concentration based on flow cytometry analysis with the use of H_2DCFDA dye and HyPer, genetically encoded probe for hydrogen peroxide, are discussed.