NADPH Oxidase Activity Controls Melanoma Cell Survival Via FAK-Src Pathway

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Reactive oxygen species (ROS) generated by NADPH oxidase (NADPHox) have emerged as important second-messengers, controling several cellular processes, such as migration, growth, proliferation and survival. Previous studies, demonstrated that NADPHox activity is essential to melanoma proliferation and survival. However, the mechanisms by which NADPHox regulates these signaling pathways have not been completely understood. In this study, we investigate the role of NADPH oxidase-derived ROS on the signaling events that coordinate melanoma cell survival. *Material and Methods* – Human melanoma cells (MV3) were incubated in absence or in presence of NADPHox inhibitor, diphenyleneiodonium (DPI, 10 µM) and melanoma cells survival was assessed by MTT assay. Confocal microscopy was used to detect actin-FAK association. Whole cell extracts were obtained for immunoblotting and imunopreciptation techniques. *Results*: We demonstrated that NADPHox inhibition reduced melanoma viability and induced changes in cellular shape, with decreased spreading and cell detachment. These events were accompanied by rearrangement of actin cytoskeleton and diminished FAK phosphorylation in Tyr^{397} residue, as well as decreased FAK association to actin and c-Src, indicating that inhibition of ROS generation would down-modulate integrin-mediated signaling, what frequently results in a particular type of apoptosis (*anoikis).* Confirming this hypothesis, we observed that DPI induced apoptosis in MV3 cells, once it triggered caspase-3 activation and DNA cleavage. Moreover, DPI effect on melanoma cells was abolished by pre-treatment of these cells with a protein tyrosine phosphatase inhibitor, sodium orthovanadate. In summary, our results suggest that ROS-generating NADPHox transmit cell survival signals on melanoma cells through FAK-Src pathway, probably inhibiting protein tyrosine phosphatase activity. The understanding of NADPHox role in tumorigenesis may lead to the development of more successful strategies to control cancer. (Supported by FAPERJ, CNPq, SR2-UERJ, ABC/UNESCO/L'Oreal)