Nitric oxide activates p21<sup>ras</sup> by S-nitrosylation leading to increase in superoxide anion levels and in cell survival

Melo, F.H.M.<sup>1,2</sup>, Molognoni, F.<sup>2</sup>, Tainah, C.S.<sup>1,2</sup>, Moretti, A.I.S.<sup>3</sup>, Souza, H.P.<sup>3</sup>, Jasiulionis, M.G.<sup>1,2</sup>

<sup>1</sup>Departamento de Micro-Imuno-Parasitologia, <sup>2</sup>Disciplina de Farmacologia, Universidade Federal de São Paulo, UNIFESP, <sup>3</sup>Departamento de Cardiologiapneumologia, Universidade de São Paulo, USP

**Introduction:** A melanocyte malignant transformation model was developed after submitting a non-tumorigenic melanocyte lineage (melan-a cells) to sequential cycles of integrin-mediated cell-matrix contact impediment. Since anoikis resistance is one of the capabilities acquired by tumorigenic cells, our aim was to investigate which alterations lead melanocytes to survive and developed tumors. In the first 3 hours of inappropriate attachment to extracellular matrix, melan-a cells showed increased superoxide anion and nitric oxide levels. Uncoupled endothelial nitric oxide synthase was identified as one of the main sources of superoxide anion during anchorage impediment, since L-sepiapterin, precursor of eNOS cofactor BH4, decreased superoxide anion levels.

**Results and Discussion:** NADPH oxidase was identified as another source of superoxide anion during anchorage blockade, since NSC, a RAC inhibitor, decreased superoxide levels. Moreover, NOX 1, but not NOX 2, 3 and 4 expressions were increased during anchorage blockade. Rac, a small Rho GTPase, is a NADPH oxidase cytosolic component that is recruited to membrane after its activation by ras. Ras signaling pathway was activated after 30 min in melan-a cells submitted to anchorage blockade. In appropriate redox environments, NO has the potential to induce the nitrosylation of cellular targets, modulating its function. Ras activation during de-adhesion was abrogated by PTIO, a NO scavenger and nitrosylation of Ras was detected, suggesting the involvement of NO in the activation of Ras signaling pathway. The maintenance of a pro-oxidant environment seems to be important in cell survival, since PTIO rendered melan-a melanocytes more sensitive to anoikis in the same way as superoxide anion depletion. PTIO decreased superoxide levels, showing its involvement in superoxide regulation production. **Conclusions:** Our results suggest for the first time in malignant transformation, that activation of p21<sup>ras</sup> by NO is a critical step in the signal transduction pathway resulting in increased superoxide production and anoikis resistance.

Supported by FAPESP and CAPES.