The role of p21Ras and the mitogen-activated protein kinases in nitric oxide-induced human monocytes apoptosis

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The monomeric protein p21Ras (Ras) is a small GTPase that cycles between an active GTP-bound form and an inactive GDP-bound form. Activated Ras can promote both, cell death and cell survival by interacting with a myriad of downstream effectors. Ras-GTP activates distinct proteins such as mitogen-activated protein kinases (MAPKs), which includes the extracellular signal-regulated kinases (ERKs), the stress activated protein kinases/c-Jun N-terminal kinases (SAPKs/JNKs), and p38 MAP Kinase. However, the kinases are differentially affected by a variety of stimuli including cytokines, growth factors, and different types of cellular stress.

Using the nitric oxide (NO) donor S-nitrosoglutathione (SNOG) and the human monocytic THP-1 cell line, we investigated the participation of Ras and the MAPKs in the NO-mediated apoptosis of THP-1 cells. Apoptotic markers such as externalization of phosphatidylserine, cell shrinkage, and chromatin condensation were evident upon cells exposure to 1 mM SNOG. THP-1 cells were permanently transfected with a Ras mutant (Ras C118S) where the critical residue for S-nitrosylation (Cys 118) was replaced by a Ser, or with wild type (wt) Ras (control for transfection). Contrasting to parental and wt-transfected cells, apoptotic signs were not evident upon exposure of Ras C118S-expressing cells to 1 mM SNOG. On the other hand, if cells were exposed to oxidative stress conditions (1 mM H$_2$O$_2$) the three cell lines underwent apoptosis indistinctly. Early activation of Ras (30 min) was observed in parental and wt cells exposed to SNOG. Ras remained inactive in RasC118S-expressing cells. Later activation (2 – 4 hs) of MAPKs was observed after incubation of cells with the NO donor. Finally, inhibition of ERK1/2 activation by the MEK inhibitor PD98059 prevented NO-mediated apoptosis. In conclusion, by stimulating the Ras-ERK1/2 MAPK signaling cascade, NO induces apoptosis in human monocytes.

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