THE LATE INCREASE OF FREE RADICALS DURING GENOTOXIC-STRESS INDUCED APOPTOSIS IS ASSOCIATED WITH CYTOCHROME C RELEASE FROM MITOCHONDRIA INDUCED BY CASPASE-MEDIATED FEEDBACK LOOP AMPLIFICATION

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Release of cytochrome c (cyt c) from mitochondria is a critical event for the onset of apoptosis induced by death stimuli. We have described recently that cyt c was released in two distinct stages and that a positive feedback loop linked caspase activation to the late cyt c release and mitochondrial dysfunction during genotoxic stress-induced apoptosis (Cell Death and Differentiation (1999) 7(2), 227-233). We show here that there are also distinct levels of intracellular reactive oxygen species (ROS), namely hydrogen peroxide and superoxide anion, measured by CM-H2DCFDA and dehydroethidium staining as determined by flow cytometry following ionizing radiation (IR) in IM-9 cells. There was a small increase of ROS and glutathione consumption in both parental IM-9 and its derivative Bcl-2 over-expressing cells following IR. The late cellular ROS increase was associated with the cyt c depletion from mitochondria by caspase-mediated feedback amplification. We showed that the late ROS production and GSH depletion were inhibited by z-VAD-fmk and ectopic Bcl-2. U266, another multiple myeloma cell line which is resistant to radiation-induced apoptosis shows no increase of ROS following IR. Furthermore, over-expression of a dominant negative caspase-9 prevented IR-induced cell death, the increase of cellular ROS levels, and GSH depletion. While ectopic Bcl-2 prevents the late ROS production and depletion of GSH, endogenous Bcl-2 could be targeted by caspases and its cleavage is associated with the late stage cyt c release and increase of cellular ROS, since over-expression of truncated Bcl-2 induces cyt c release, and consequently apoptosis. These results suggest that intracellular ROS production is associated with the late cyt c release and that Bcl-2 truncation is a component of the amplification loop of caspase-mediated cyt c release and cellular ROS production.
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