A NOVEL APOPTOSIS-LIKE CELL DEATH, INDEPENDENT OF CASPASE-3, INDUCED BY CURCUMIN

EWA SIKORA
Laboratory of Molecular Bases of Aging, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Apoptosis is currently the subject of intense research interest, partially because we now recognize that tumor cells can die by apoptosis in response to cytotoxic treatments with drugs or radiation. Apoptotic cells have well-defined characteristics to this mode of cell death: specific DNA digestion, chromatin condensation, and morphological changes such as actin-dependent phenomena of cytoplasmic blebbing. Curcumin, a yellow pigment from rhizome of Curcuma longa currently undergoing clinical evaluation, has been shown to induce apoptosis in numerous animal and human cells, although the cell death pathway depends very much on cell type. We studied the ability of curcumin to induce cell death in many human and rodent transformed as well as normal cells (HL-60, Molt 4, Jurkat, T cells, splenocytes, thymocytes, L1210). Normal cells were either quiescent or stimulated to proliferate. We showed that 50 μM curcumin is able to induce cell death in all studied cells, but cell death symptoms varied for different cells. All of them died as assessed by TUNEL method or trypan blue exclusion test as well as morphological observation. No type of cells showed oligonucleosomal DNA fragmentation (DNA “ladder”) as effect of curcumin action, although in HL-60 cells, we were able to observe sub-G1 formation and caspase-3 activation. These data show that in all tested cells curcumin induces cell death which can be classified as apoptosis-like, and only in HL-60 cells it can be recognized as classical apoptosis. In cells which undergo non-classical apoptosis curcumin can be characterized by a dual mode of action. It induces non-classical apoptosis via still unrecognized mechanism preventing classical symptoms of cell death, namely mitochondrial membrane potential decrease, Bcl-2 protein level decrease, caspase-3 activation and internucleosomal DNA fragmentation. In these studies we concentrated on the absence of classical symptoms of curcumin-induced apoptosis in Jurkat cells. Searching for a possible mechanism responsible for this feature, we focused our attention on glutathione as the main cell antioxidant and on its role in programmed cell death. Reactive oxygen species have been shown to induce apoptosis as well as mediate cell death induced by numerous different factors. Although some controversy about involvement of reactive oxygen species in apoptosis exists, the obvious drop in glutathione content in cells undergoing apoptosis can not be neglected. Moreover, glutathione depletion either causes apoptosis or can at least sensitize cells to death. In Jurkat cells complete depletion of glutathione did not induce apoptosis, but it sensitized them to undergo apoptosis with some classical
symptoms, namely caspase-3 activation and DNA fragmentation after curcumin treatment. Therefore, the reason of curcumin protection against caspase-3 activation was probably the lack of glutathione decrease whose level was even higher (130%) in curcumin-treated than in control cells. The question which is still open is how curcumin induces glutathione elevation. Some data exist proving that it can be via Bcl-2. Bcl-2 is the main antiapoptotic protein whose decreased level is tightly connected with cytochrome c release, caspase-3 activation and final cell death. We demonstrated that curcumin, even within glutathione-depleted cells, did not change Bcl-2 level. Altogether, we assume that the role of curcumin in prevention of pro-caspase-3 proteolysis relays on protecting against glutathione drop possibly by preventing Bcl-2 decrease. However, this mechanism cannot be applied to other cells which, when treated with curcumin (at the same concentration), undergo classical apoptosis (for example HL-60). A possible explanation of this discrepancy and the role of glutathione in cells with different response to curcumin is under evaluation.