INTRACELLULAR SIGNALING MECHANISMS IN STAUROSPORINE-INDUCED APOPTOSIS IN HUVEC

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Apoptosis is an active and highly regulated program of cell death involving a complex cascade of events, but liable for a common pattern of morphological alterations: chromatin condensation, DNA cleavage, mitochondria breakdown and membrane blebbing that cause the decline of the cell into the typical apoptotic bodies. Many studies have proved the crucial role of the Bcl-2 family of proteins in the regulation of the intracellular pathways leading to apoptosis. While some members of this family have been shown to promote cell survival such as Bcl-2, Bcl-XL, Mcl-1 and A1, others like Bax, Bad, Bim and Bcl-Xs, have exhibited pro-apoptotic effects. It seem now clear that the relative levels of the two groups can influence the susceptibility of the cell to undergo apoptosis, even if the exact biochemical effect of each member is not completely understood. In the absence of death signals, many of these homologues proteins are localized in different subcellular compartments. The anti-apoptotic members, such as Bcl-2 are present into the mitochondria, nuclear envelope and endoplasmic reticulum. Also Bad, promoter of cell death, can be integrated into mitochondrial outer membranes, but most of the pro-apoptotic proteins in their physiological inactive form are localized into the cytosol. The main characteristic of this family consists on the ability to modulate their own activity by post-translational modifications such as dimerizations, proteolytic cleavage and also phosphorylation of Bcl-2 (Gross, 1999). The upregulation of this protein can contribute to the cell survival. On the contrary, its downregulation or the formation of a complex with Bad promotes the activation of the apoptosis. The first consequence is the release of cytocrome c into the cytoplasm, the activation of the apoptosome, a complex of procaspase-9 and APAF-1 which is at the head of a complex hierarchy of proteinases directly responsible of the cell death and well known as the caspase cascade. Many studies have suggested that the upregulation of the anti-apoptotic factors of Bcl-2 family, particularly the Bcl-2 protein, can guarantee mitochondrial integrity and so prevent the activation of the apical caspases responsible of the cytocrome c mediated apoptotic pathway. The Bcl-2 expression can be modulated positively by colturing many types of cell lines, especially the endothelial ones, in the presence of serum basic fibroblastic growth factor (bFGF) and vascular endothelial growth factor (VEGF), two multifunctional cytokines responsible for cell mytogen and proliferation. In fact, it has been demostrated both VEGF and bFGF are able to protect serum-starved human umbelical vein endothelial cells (HUVEC) from apoptosis acting as survival factors by the increase of Bcl-2 and A1 levels (Gerber, 1998).
Recently, experiments of our research group have shown the ability of VEGF and epithelial growth factor (EGF) to slow down apoptosis in HUVEC induced not only by serum starvation but also by staurosporine, well-known apoptotic agent. In fact, MTT essays on cell viability and enzymatic measurements of caspase-3 levels show that VEGF and EGF antagonize the staurosporine-induced apoptosis in HUVEC (Vinci et al. 2002). To understand the signaling mechanisms of the anti-apoptotic effect of VEGF, the relationship between VEGF, staurosporine and Bcl-2 expression in HUVEC has been studied. The cells were isolated and grown up according Jaffe's method (1973) and used between passage two and six. For the experiments, HUVEC ($1.5x10^6$) were cultured in 100-mm dishes initially in endothelial basal medium and, the following day, in fresh serum free medium. Western blotting analysis with whole cell extracts exposed to VEGF and VEGF plus staurosporine has been performed in order to evaluate the expression of the Bcl-2 family proteins, and in particular the anti-apoptotic members Bcl-2 and Mcl-1. The results indicate that Bcl-2 is expressed in HUVEC and the expression is enhanced by VEGF and reduced by the apoptotic agent staurosporine. Experiments are in progress in our laboratory to relate the Bcl-2 family proteins to the signaling mechanisms of VEGF protective effect on iatrogenic apoptosis.

References

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