MOLECULAR MECHANISMS OF APOPTOSIS

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Apoptosis is a form of physiological cell death that functions to control cell populations during such process as embryogenesis, immune responses and many other examples of normal tissue homeostasis. Although most forms of apoptosis culminate in the internucleosomal cleavage of DNA, the biochemical basis of signal transduction is poorly understood. Furthermore, it is now clear that different pathways leading to DNA fragmentation may be activated in responses to different stimuli.

Efforts in our laboratory to elucidate the biochemical basis of apoptosis have focused primarily on the human U937 histiocytic lymphoma as a model system. This cell line readily undergoes apoptosis in response to a variety of stimuli including tumor necrosis factor alpha (TNF), UV light, chemotherapeutic drugs, heat shock, and antibodies directed against the Fas antigen. TNF and UV light are specially rapid and potent inducers of DNA fragmentation in this system, in that within 1.5-3 h of exposure to these agents, it is possible to obtain populations in which virtually 90-100% of the cells are undergoing apoptosis. The availability of relatively homogeneous apoptotic cell populations greatly facilitates the identification, isolation and characterization of enzymes involved in this response.

Thus far, our findings suggest that both TNF and UV light activate overlapping or identical pathways leading to DNA fragmentation in U937 cells. The response to both stimuli is independent of extracellular calcium (unpublished observations) or protein synthesis. DNA fragmentation is suppressed by a protein kinase inhibitor selective for myosin light chain kinase, KT5926 and by the PKC activator, PMA. Furthermore, both TNF and UV light-induced apoptosis is inhibited by the ADP-ribosyl transferase inhibitor, 3-aminobenzamide. Resistance to apoptosis may play a role in the process of carcinogenesis and recent studies from this laboratory demonstrate that 10/10 tumor promoters block TNF, UV-light and chemotherapeutic drug induced apoptosis is a variety of cell lines.

Although the mechanism of TNF signal transduction is not completely understood, recent studies have shown that one of the earliest cell responses is activation of a neutral sphingomyelinase (SMase) which generates ceramide that is thought to act as a second messenger. TNF rapidly activated sphingomyelin hydrolysis in U937 as other cells sensitive TNF-induced apoptosis (9). Furthermore, addition of exogenous cell-permeable ceramide or bacterial-derived sphingomyelinase induced DNA fragmentation in target cells. The observation that TNF activates sphingomyelin hydrolysis, even in a cell free system, suggested that SMase activation is tightly coupled to the TNF receptor.

Recent results a defect in the activation of SMase appears to be responsible for the resistance of the U93-TR variant to TNF or UV light-induced DNA fragmentation.

REFERENCES