Cleavage of DNA Replication Proteins Mcm3 and Cdc6 and Differential Gene Expression in B Cell Apoptosis

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ABSTRACT

Apoptosis, a conserved cellular suicide program, exhibits important functions in the development, differentiation and homeostasis of tissues and organs in all multicellular organisms. In the present study, a profound downregulation of proliferation after induction of apoptosis in the Burkitt's lymphoma cell line BL60-2 was detected and consequently the impact of apoptosis on the pre-replicative complex (pre-RC), which is essential for the initiation of DNA replication, was analyzed. A reduction on mRNA level for the pre-RC components Mcm2 – Mcm7 and Cdc6 early in the apoptotic process was revealed. In addition, an apoptotic cleavage for two of the corresponding proteins, Mcm3 and Cdc6, was detected. The cleavage of both proteins seems to be a general phenomenon in apoptotic cells, as it was detected in three distinct cell lines and in response to diverse apoptotic stimuli.

The apoptotic cleavage of Mcm3 and Cdc6 is mediated by caspases. The involved effector caspases as well as the respective cleavage sites were determined for both proteins *in vitro*. Mutation of the identified cleavage sites in Mcm3 and Cdc6 generated proteins, which were not cleaved upon the overexpression and subsequent induction of apoptosis in HeLa cells. The cloning and overexpression of the apoptotic Mcm3 and Cdc6 fragments in HeLa cells allowed the analysis of functional characteristics. A pro-apoptotic effect was revealed for the N-terminal Mcm3 fragment, indicating a possible function for Mcm3 in a positive feedback loop in apoptotic cells.

Apoptosis is an active process depending on the *de novo* expression of specific proteins. On the other hand, anti-apoptotic proteins have to be repressed. To investigate the differential expression of genes in apoptotic compared to normal BL60-2 cells, the respective mRNA preparations were analyzed on Affymetrix GeneChips, cDNA membranes, and in RNase protection assays. Above all, a significant decrease on mRNA level for six genes involved in DNA repair was identified in apoptotic compared to non-apoptotic cells. Two of the corresponding proteins are also enzymatically cleaved in apoptosis, indicating that the shutdown of DNA repair is important for apoptotic cells.

Taken together, the downregulation of important components of DNA replication and DNA repair in apoptotic cells on protein and mRNA level was shown. The downregulation of futile DNA replication and repair probably enables an unobstructed apoptotic DNA fragmentation and prevents the waste of ATP, which is important, as ATP-depletion was shown to change apoptotic to necrotic cell death with its unfavorable side-effects like inflammation. In addition, a pro-apoptotic effect for an apoptotic Mcm3 fragment was revealed. By the induction of an amplification step, the caspase-mediated cleavage of pre-RC components might fulfill an important function in the apoptotic cell, exceeding the inhibition of DNA replication.

INDEX