Anti-Apoptosis Engineering for Mammalian Cell Culture
Using 30K Protein and Its Gene Originating from Bombyx mori

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We have reported that silkworm hemolymph inhibits apoptosis induced by various methods in insect, mammalian and human cell systems. To isolate the apoptosis-inhibiting component from silkworm hemolymph, it was fractionated by column chromatography, and each fraction tested for anti-apoptotic activity. A database search, using the N-terminal amino acid sequence of the protein with the highest activity as a template, resulted in a 95% homology with one of the ‘30K proteins’. The 30K proteins are a specific type of plasma protein called ‘storage proteins’; they are a group of structurally related proteins, with molecular mass of approximately 30,000 Da. The cDNAs coding for the 30K proteins were constructed and one of the cDNAs, 30Kc6, was introduced into mammalian cells to develop the apoptosis-resistant cell line. The 30K protein was expressed in HEK293 and CHOK1 cells by transfection with the vector containing the 30Kc6. The HEK293 cell apoptosis, induced by staurosporine, was inhibited when the 30K protein was transiently expressed. In the case of CHOK1 cell lines expressing the 30K protein, an apoptosis-inhibition and a lower intracellular activity of caspase 3 were observed. This indicates that apoptosis is inhibited when the 30K protein is expressed in the cells. Consequently, this apoptosis-resistant CHO cell line increased the production of recombinant EPO. Anti-apoptosis engineering, using the 30K protein and the corresponding gene, will provide a new anti-apoptosis strategy for therapeutic approaches and industrial applications of animal cell cultures, and it can be also used to inhibit the cell death which frequently occurs in animal cell culture on microfabricated chips

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