6 Summary

In the work presented here, the essential involvement of caspases in the cell death of AKR 2B-fibroblasts could be proved as well as their activities could be characterized.

Confluent AKR 2B-fibroblasts, a good characterized and subcloned cell line, rapidly disintegrate after serum deprivation. Dying of the cells ceases after 6 hours with a survival of 50%. These surviving cells remain uneffected for additional 48 hours which is dependent on neo-protein biosynthesis. During cell death AKR 2B-fibroblasts show morphological changes characteristically for apoptosis, even though typical features like oligonucleosomal DNA fragmentation is absent.

Using different approaches the expression of mRNA of all known caspases, which are believed to be involved in apoptosis essentially, was successfully detected in AKR 2B-fibroblasts. Caspases-1, -2, -3, -6 and -9 were constitutively expressed as zymogens. With the exception of Caspase-9, the processing into their active subunits induced by serum removal could not be detected. At least their considerable importance during cell death of AKR cells could be proved by using specific caspase inhibitors and by determination of their specific activity. The characterization of that activity gave some hints for the identity of activated caspases. Beside constutive VEIDase and IETDase activities, a DEVDase reaches its maximum 3 hours after the onset of apoptosis. The present mixture of caspase activity is dominated by this DEVDase, which seems to be represented by just one enzyme, as shown by affinity labeling and 2D-SDS-PAGE. Determinations of K_M- and K_i-values lead to the conclusion, that this enzyms has typical effector caspase characteristics, like caspase-3. Cleavage of lamins during cell death of the fibroblasts indicate that a caspase-6 became active. However, the known characteristics of caspase-6 are different of that found in AKR 2B cells, so that it may play just a minor role in the caspase mixture. Established repeated purification steps by chromatography, offers best conditions for protein sequencing and identification of the active caspase.

The involvement of the receptor mediated pathway could be excluded by an overexpression of CrmA , a cowpox virus derived Caspase inhibitor; also there are no hints for an involvement of the mitochondria mediated pathway, except of caspase-9 cleavage. Pathways which lead to DEVDase activation are of major interests in present and future. Stimulation of signal pathways by PDGF-BB, TPA, Forskolin and 8Br-cAMP and others agents protect the fibroblasts from death. The stimulated pathways converge in one point up stream of effector-caspase activation. The identification of this point and its regulatory properties is a future goal, which will maybe lead to an understanding of processes responsible for surviving of