7. Summary

This thesis aimed to elucidate the pathways in apoptotic signalling that are activated by exogenous expression of the proapoptotic mitochondrial protein Smac/DIABLO. Besides this the potential usage of exogenous Smac-expression for sensitisation of malignant cells for cytostatic drugs was investigated. To this aim, the full length human Flag-tagged Smac cDNA was cloned into an adenoviral vector which allows for the conditional expression of the transgene under the control of a Tet-Off-System *in cis*. Correct processing of the 29 kDa Smac precursor into its 23 kDa mature form was shown by western blot analysis. Immunofluorescence staining showed mitochondrial localisation of the Smac protein.

The enforced expression of Smac induced apoptotic DNA-fragmentation as well as in HCT116 $Bax^{+/-}$/$Bax^{-/-}$ colon carcinoma cell lines and Bax negative DU145 mock and Bax reconstituted prostate carcinoma cell lines. Induction of apoptosis occurred irrespective of their expression status of the proapoptotic Bcl-2 homologue Bax. Detection of DNA-fragmentation was paralleled by cleavage of caspases-3, -8 and -9. The addition of a broad range caspase-inhibitor completely abrogated Smac-induced cell death. Mitochondrial activation was induced independently from Bax. Regarding the release of cytochrome c, a limited dependence from Bax was observed in DU145 Bax cells that could be suppressed by the use of a broad range caspase-inhibitor.

To further investigate the influence of the antiapoptotic Bcl-2 homologue Bcl-x_L during Smac-induced apoptosis, stable HCT116 Bcl-x_L transfectants were generated by the use of a retroviral vector. Overexpression of Bcl-x_L suppressed apoptosis induced by the anticancer drug epirubicine, whereas cell death induction by Smac was not affected.

To address a putative role of Smac at the endoplasmic reticulum, calcium effluxes from the endoplasmic reticulum into the cytosol were assessed. An increase in cytosolic calcium levels was readily detectable and preceded an activation of the mitochondria. In comparison to the Bax-deficient cells the Bax expressing DU145 cell line showed a stronger induction of calcium efflux after treatment with Smac and thapsigargin.

Finally caspase-3 was shown to be the dominant target of Smac. Caspase-3 deficient MCF-7 breast cancer cells proved to be completely resistant, while caspase-3 reconstituted MCF-7 cells were susceptible to Smac-provoked apoptosis. Moreover, the combined treatment of adenoviral expression of Smac and subtoxic doses of the anticancer drugs epirubicine and etoposide resulted in a considerably higher amount of apoptotic cell death with a clear dependency on caspase-3.

It could be demonstrated that the expression of exogenous Smac-protein induced apoptosis in different carcinoma cells, besides their defects in the mitochondrial apoptosis signalling pathway upon the loss of Bax or the overexpression of Bcl-x_L. Moreover, Smac mediated a sensitisation of tumour cells for cytostatic drugs. Therefore Smac can be regarded as a novel therapeutic approach to overcome resistant malignant phenotypes.