Summary

Constitutive and acquired resistance to cytostatic drug-induced cell death is the major obstacle for the cure of malignancies. It was therefore interesting to test whether overexpression of death promoting factors can overcome resistance to cytotoxic therapies.

Two model systems of drug-sensitive, MT-1 and MT-3, and drug-resistant breast cancer cell lines, MT-1/Adr and MT-3/Adr, were used to investigate whether the pro-apoptotic Bcl-2 homologues Bak or Nbk/Bik can overcome resistance for drug-induced apoptosis.

The following results could be achieved:

1. First, Bak and Nbk/Bik stable transfectants were generated in MT-1/Adr and MT-3/Adr cells.

2. The defect in the MT-1/Adr cells is associated with strongly increased Bcl-2 expression. In contrast, the MT-3/Adr cells overexpress the Mdr-1/p-glycoprotein and drug resistance is due to an increased detoxification by removal of the drug from the cell by the Mdr-1 ABC transporter.

3. In the MT-1/Adr cells, the overexpression of Nbk/Bik sensitized the MT-1/Adr cells for epirubicin-induced apoptosis and partially antagonized drug resistance. The overexpression of Bak, however, completely reversed the resistant phenotype in the MT-1/Adr cells and conferred sensitivity to epirubicin-induced apoptosis which was equivalent to the original, drug-sensitive MT-1 cells. In the MT-3/Adr cells, overexpression of both Bak or Nbk/Bik enhanced drug sensitivity for epirubicin-mediated apoptosis as compared with the MT-3/Adr mock transfectants. In contrast, etoposide- and taxol-mediated apoptosis could not be influenced by overexpression of Bak or Bik/Nbk. This observation has led to investigations of functional differences between epirubicin and etoposide-induced apoptosis. There were no difference in the amount of free reactive oxygen species (ROS) during epirubicin-induced apoptosis between sensitive and resistant cells. Nevertheless, etoposide-sensitive cells produced more ROS than etoposide-resistant maternal cells and the Bak or Bik/Nbk transfectants.

4. Overexpression of both Bak and Nbk/Bik was capable of reverting the defective mitochondrial activation after exposure to epirubicin. A similar sensitizing effect was

observed when caspase-3 activation was analyzed. Both Bak and Nbk/Bik transfectants showed a strong induction of caspase-3 activity when exposed to epirubicin.

5. In order to investigate whether Bak may also revert resistance against heat-shock (thermoresistance), the protein was stably expressed in the thermoresistant gastric cancer cell line EPG 85/257/T. Measurement of apoptotic DNA fragmentation showed that heat shock induces apoptosis via a bcl-2-sensitive mitochondrial signalling pathway. Thus, the overexpression of Bak could sensitize the thermoresistant cells to heat-shock-induced apoptosis.

Altogether, these data show that the manipulation of the downstream apoptosis signalling cascade by the overexpression of Bak or Nbk/Bik can overcome not only drug resistance due to mitochondrial apoptosis deficiency (in the MT-1/Adr cells) but also classical, i.e. efflux-mediated, resistance for drug-induced cell death in the MT-3/Adr cell line. Finally, the fact that both Nbk/Bik and Bak sensitize tumor cells for drug- and heat-shock-induced apoptosis and thereby may overcome resistance suggests that the manipulation of such apoptosis promoting Bcl-2-like proteins might yield a therapeutic strategy to overcome drug resistance in tumors refractory to cytotoxic therapies.