

6. SUMMARY

A decisive characteristic of cancer cells is their apoptosis deficiency due to which uncontrolled growing cells can not be eliminated. At the mitochondrial level, members of the Bcl-2 family represent important regulators of apoptosis. The melanoma cell lines, investigated, exhibited high expression of the antiapoptotic proteins Bcl-2 and Bcl-X_L as compared to cultures of normal human melanocytes, whereas the proapoptotic Bcl-X_S was not detectable at the protein level. Melanoma cell lines showing resistance against agonistic CD95 activation (CH-11) and against ceramide were characterised by a high ratio of Bcl-2 / Bax. The relevance of Bcl-2 proteins was confirmed in two melanoma cell lines sensitive for CH-11 and ceramide, in which stable overexpression of Bcl-2 caused resistance against apoptosis stimuli.

In order to address the role of Bcl-X_S and Bcl-X_L in the regulation of apoptosis in melanoma cells, both genes were cloned by RT-PCR and were integrated into a tetracycline-regulatable expression plasmid system. During cloning, a novel splice variant of Bcl-X with a molecular weight of 18 kDa and a still unknown function was also identified. The induction of Bcl-X_S in stably transfected melanoma cell clones resulted in a significant increase of apoptosis, whereas overexpression of Bcl-X_L diminished basic apoptotic levels.

Investigating the mechanism of Bcl-X_S-mediated apoptosis, Bcl-X_S overexpression was found to reduce the level of cytochrome c in mitochondria, but increased cytoplasmic levels of cytochrome c were not detectable. Also, activation of caspase-3 and -8 was only weak and transient. Interestingly, after Bcl-X_S induction a release of the apoptosis inducing factor (AIF) was detected, which displays its proapoptotic effect independently of caspases. These results suggest that Bcl-X_S mediates apoptosis without involvement of caspases. The proapoptotic effects of different cytostatic agents and other proapoptotic stimuli could be significantly increased by overexpression of Bcl-X_S. Investigating the relevance of Bcl-X_S overexpression *in vivo*, significantly delayed tumor growth was found after induction of Bcl-X_S in a nude mouse model resulting in 4.4-fold smaller tumors.

The present study proves the importance of the ratio of proapoptotic to antiapoptotic proteins of the Bcl-2 family for apoptosis sensitivity in melanoma cells. The promising results in the animal model may suggest a therapeutic approach based on Bcl X_S.