5. SUMMARY

Malignant melanoma of the skin is characterized by unbroken high mortality and increasing incidence rates in recent decades. Of major importance is a high metastatic potential as well as apoptosis resistance which substantially contributes to immune escape mechanisms and to inefficiency of chemotherapeutics. Thus, new therapeutic strategies are urgently needed to improve the dismal prognosis of this disease.

Apoptosis is a well defined genetic death program leading to ordered destruction of cellular components while membrane integrity is maintained. Two major pathways have been described which activate aspartate-specific cysteine proteases (caspases) representing major effectors in realization of apoptosis. The intrinsic pathway, also activated by chemotherapeutic drugs, depends on intracellular signals such as DNA damage, mitochondria and the tumor suppressor p53. On the other hand, the extrinsic pathway is initiated by specific binding of death ligands to cell surface death receptors and formation of the death-inducing signaling complex (DISC), which triggers activation of the caspase cascade.

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL/APO2L) belongs to the TNF superfamily and binds to its death receptors TRAIL-R1/DR4 and TRAIL-R2/DR5. This results in receptor oligomerization, DISC formation and subsequent activation of initiator caspases -8 and -10. Effector caspases such as caspase-3 are either directly activated by caspase-8, or this caspase cleaves and activates the proapoptotic Bcl-2-related protein Bid, which delivers the signal further downstream to the mitochondria and thus connects the two major pathways.

The ability of TRAIL to induce apoptosis in a wide variety of cancer cell lines, while having only little toxicity for most types of normal cells, suggests that this molecule might be very useful for future cancer therapies. Preclinical studies already demonstrated the high potential of TRAIL in mice and in primates, when used as a single agent or in combination with chemotherapeutic drugs. Suppression of tumor xenografts without obvious signs of systemic toxicity qualified TRAIL as a promising strategy for cancer therapy. Clinical trials with TRAIL or agonistic monoclonal antibodies for its receptors have already been initiated for some solid tumours so far, however, excluding melanoma.

In this study, the expression and functionality of DR4 and DR5 in melanoma cell lines in vitro as well as their significance for the in vivo situation was investigated. Mechanisms of TRAIL-resistance have been addressed by investigation of TRAIL-induced NF-κB activation and by establishing a cell culture model for TRAIL-resistance.

DR5 was found consistently expressed in all melanoma cell lines, whereas DR4 expression was found only in 2/7 cell lines. High sensitivity to TRAIL-induced apoptosis was characteristic for DR4-positive melanoma cells, whereas DR4-negative cell lines showed less and delayed response or were completely resistant. Employment of selective DR4/DR5 blocking antibodies unequivocally proved the prevalent role of DR4 in the melanoma cell lines, where it was expressed. The significance of these data for the in vivo situation was finally evaluated by immunohistochemistry, which proved for the first time pronounced expression of DR4 as well as of DR5 in melanoma primary tumors.

Apoptosis resistance, however, is a major problem of almost any cancer therapy. In several investigations, resistance to TRAIL-induced apoptosis was correlated with the basic expression of antiapoptotic proteins such as c-FLIP, Bcl-x_L, Bcl-2 and IAP family members. Transcription factor NF-κB has been suggested as critical for TRAIL resistance, as NF-κB-dependent expression of Bcl-x_L, c-FLIP, XIAP may serve to protect cancer cells from TRAIL-induced apoptosis. Along with other TNF ligands, TRAIL has been shown to trigger NF-κB activation.

In this study, high TRAIL-induced activation of NF-κB was seen only in apoptosis sensitive melanoma cell lines expressing DR4, whereas TRAIL failed to activate NF-κB in DR4-negative (DR5-positive) melanoma cells regardless their sensitivity. Activation of NF-κB by TRAIL was not associated with any changes in the expression of the antiapoptotic proteins as c-FLIP, Bcl-x_L, XIAP, Survivin or Livin. Rather, TRAIL induced downregulation of DR4 in one of the cell lines, and this effect was prevented by proteasome inhibition (LLnL). In general, LLnL increased TRAIL sensitivity in melanoma cell lines was independent from NF-κB activation.

Also in selected, TRAIL-resistant melanoma cells, neither an increase in TRAIL-induced NF-κB activation nor an increased expression of antiapoptotic proteins was found. Selected TRAIL-resistant melanoma cells, which were DR4-positive, restored their sensitivity after TRAIL withdrawal from culture medium after one month, whereas DR4-negative cells remained resistant even after a longer period of culturing in the absence of TRAIL.

Unraveling the mechanism of TRAIL resistance in melanoma cell lines, significant downregulation of the expression of caspase-8, caspase-10 and of DR4 but not of DR5 was found in selected, TRAIL-resistant melanoma cells. Sensitivity of selected, TRAIL-resistant melanoma cells was largely retained after exogeneous overexpression of initiator caspases and of DR4.

Thus, high efficiency of DR4-mediated apoptosis in vitro and frequent DR4 expression in vivo may suggest reassessment of the suitability of TRAIL and especially of DR4-based strategies for melanoma treatment. Resistance to TRAIL-induced apoptosis in melanoma cells may be based on downregulation of initiator caspases and of DR4 but seems to be unrelated to NF-κB-driven upregulation of antiapoptotic factors.

Expression of DR4 and caspases seems clear to support sensitivity. Levels of these factors may be increased by proteasome inhibition, which prevented TRAIL-induced downregulation of DR4 (shown here), and by chemotherapeutics, which may upregulate TRAIL death receptors or by interferon-γ, which was shown to upregulate initiator caspase-8. Transient resistance of melanoma cells may also be circumvented by temporary interrupted administrations.

Finally, as current chemotherapies of malignant melanoma in its metastatic stage have achieved only limited effect, new biological agents are interesting. TRAIL can offer this promis as it selectively induces apoptosis in cancer cells, while sparing normal cells. However, because of discrepancies in the literature, the potential of TRAIL-based strategies for malignant melanoma was not fully recognized. This theses may contribute to the understanding of the nature of TRAIL-induced apoptosis and resistance in melanoma cells, and therefore supplies practical oncologist with valuable information supporting future TRAIL therapy in patients with malignant melanoma.