4. DISCUSSION

4.1. High significance of DR4 expression in melanoma cells

4.1.1. Consistent expression of DR5 but limited of expressionDR4

The compelling advantage of TRAIL is that it triggers apoptosis in a variety of human cancer cells, whereas normal cells and tissues were largely spared (Yagita *et al*, 2004), is highly suggestive for employing TRAIL in experimental tumor therapies. The tumor toxic effect requires selective binding of TRAIL to one of its agonistic death receptors (DR4 or DR5) and thus is highly dependent on their functionality in the target cell. An increased expression of agonistic TRAIL receptors in cancer cells has been attributed to their higher sensitivity (Ryu *et al*, 2000; Koornstra *et al*, 2003). DR5 was found in various tumor cells, but DR4 was often lacking in resistant cells (Kim *et al*, 2000). For melanoma, the majority of cell lines were reported as positive for DR5 whereas, DR4 was often lost (Zhang *et al*, 1999; Zhang *et al*, 2000a).

In agreement with previous data, we showed here consistent surface expression of DR5 in seven melanoma cell lines and in cultures of normal human melanocytes whereas, significant expression of DR4 was found only in two of the melanoma cell lines, as determined by FACS analysis. Comparison of the surface expression data with Western blot analyses from total protein extracts revealed protein bands for DR5 largely in agreement with previous reports on hepatocellular carcinoma, lymphoma and Jurkat cells (Bodmer *et al*, 2000; Higuchi *et al*, 2001). For DR4, however, the situation was less clear, as protein bands of highly varying size had been reported, in particular of roughly 57 kDa (Higuchi *et al*, 2001; Matysiak *et al*, 2002; Song *et al*, 2003). For our panel of melanoma cell lines, we found only weak signals at this molecular weight, which furthermore did not show any correlation to the FACS expression data. However, correlation to FACS analysis was seen for a 44 kDa protein, strongly suggesting that this protein may correspond to DR4 in melanoma cells. A protein of similar molecular weight has also been reported in HeLa cells (Lichtenstein *et al*, 2004). For the first time, we have proven the identity of the 44 kDa protein of DR4 and of the 47 kDa protein of DR5 for melanoma cells by transient transfection of the respective cDNA plasmids. As the 44 kDa band of DR4 was in question, after these experiments it became clear that this protein indeed corresponds to DR4 in melanoma cells.

4.1.2. High TRAIL-induced apoptosis in melanoma cells positive for DR4

Targeting of tumors by TRAIL depends on the functionality of the respective death receptors. However, a clear distinction between the roles of two agonistic receptors (DR4 and DR5) has yet to be defined. Functional differences between two receptors may be assumed with respect to apoptosis induction. Both agonistic receptors might equally contribute to TRAIL-mediated apoptosis in certain cells. However, due to correlation between expression and sensitivity data or due to applying selective recombinant TRAIL or selective antagonistic antibodies, the prevalent role of either one or the other death receptor has been proposed. For untransformed cells as for fibroblasts, a prevalent role of DR5 has been postulated (Ichikawa *et al*, 2003), whereas TRAIL sensitivity was proven to result mainly from DR4 in oligodendrocytes and in keratinocytes (Matysiak *et al*, 2002; Leverkus *et al*, 2003).

For tumor cell lines, several reports suggested a major contribution of DR4 in cells from different origin as for lung, colon, breast carcinomas and for lymphoma, Ewing's sarcoma tumors (Kim *et al*, 2000; Mitsiades *et al*, 2001; Strater *et al*, 2002; Kazhdan and Marciniak 2004; Jin *et al*, 2004b; MacFarlane *et al*, 2005). Also, primary cells from patients with chronic lymphocytic leukemia and mantle cell lymphoma were selectively killed by specific agonistic antibodies directed against DR4 but not against DR5 (MacFarlane *et al*, 2005). The expression of DR4 but not of DR5 in colon cancers was linked to a favourable prognosis (Strater *et al*, 2002).

According to studies with TRAIL mutants that specifically bind to one or the other receptor, a leading role of DR5 has also been suggested in TRAIL-induced apoptosis (Kelley *et al*, 2005). On the other hand, for primary tumors from non-small cell lung cancers, detection of DR5 expression by immunohistochemistry was associated with an increased risk of patients death (Spierings *et al*, 2003).

Also for melanoma cells and normal melanocytes, especially DR5 has been regarded as critical for TRAIL-induced apoptosis (Zhang *et al*, 1999; Zhang *et al*, 2000b; Hersey and Zhang 2001; Zhang *et al*, 2004b). This may partly result from the fact that several TRAIL-sensitive melanoma cell lines were characterized by high levels of DR5. However, no clear correlation between sensivity and expression levels of DR5 could be found (Zeise *et al*, 2004), which may be indicative for a high impact of intracellular regulation mechanisms such as reported for cFLIP, Bcl-2 proteins, inhibitors of apoptosis (cIAPs) and NF-κB activation (Hussein *et al*, 2003).

Functional studies on the contribution of DR4 in melanoma, however, are spare. Only two melanoma cell lines have been submitted to functional analyses applying specific DR4 blocking antibodies, as reported in the literature. In one cell line, this treatment remained without effect (Griffith *et al*, 1999). The reduction of TRAIL sensitivity observed in the other cell line was attributed to DR4-mediated downregulation of TRAIL decoy receptors (Zhang *et al*, 2000a). The significance of decoy receptors for regulation of TRAIL sensitivity in melanoma cell lines, however, remains elusive (Zhang *et al*, 1999; Zeise *et al*, 2004). Here, we found that melanoma cell lines positive for DR4 in addition to DR5, as characterized unequivocally by FACS analysis, immunocytochemistry and Western blot analysis, revealed highest sensitivity to TRAIL-induced apoptosis already after 6 h, whereas melanoma cell lines lacking DR4 showed reduced and time-delayed sensitivity or remained largely resistant. DR4-positive melanoma cell lines did not show any signs of cytotoxicity in the early stages of TRAIL treatment (6 h), indicating that the primary effect of TRAIL was apoptosis in these melanoma cell lines.

Clear indication of activation of apoptosis cascade, in particular, activation of caspases, was seen in all sensitive melanoma cells. In the course of TRAIL-induced apoptosis, only the mature p17 cleavage product of caspase-3 (Han *et al*, 1997) correlated with TRAIL sensitivity, whereas the intermediate product p20 observed in all – sensitive and resistant – melanoma cell lines. This might indicate that some caspase-3 cleavage activity was also induced in resistant cells, but the final step leading to mature caspase-3 seemed to be restricted to sensitive cells. Selective cleavage of p17 has also been reported for another TRAIL-sensitive melanoma cell line (Zhang *et al*, 2001).

Degradation of the caspase inhibitory protein XIAP has been regarded as critical for TRAIL-induced apoptosis in melanoma (Zhang *et al*, 2001). Cleavage products of XIAP were also seen in the present investigation early after TRAIL treatment, however, cleavage was not sufficient to significantly reduce basic XIAP levels after 4 h. Thus for early TRAIL-mediated apoptosis (after 6 h), degradation of XIAP seemed to be less responsible in melanoma cells.

In agreement with previous findings regarding the significance of the mitochondrial pathway for CD95/Fas-mediated apoptosis in melanoma (Raisova *et al*, 2001; Eberle *et al*, 2003), clear characteristics of the mitochondrial pathway were also seen in A-375 upon TRAIL-mediated apoptosis, namely, cleavage of caspase-9 and Bid. Furthermore, apoptosis was completely blocked in A-375 and Mel-HO after stable overexpression of Bcl-2. On the other hand, TRAIL-induced apoptosis was only partially blocked in SK-Mel-13 after transient overexpression of Bcl-2 or after stable overexpression of Bcl-x_L. The fact that SK-Mel-13

revealed no cleavage of BID as well as an only weaker cleavage of caspases may be seen as indication for additional alternative pathways also in TRAIL-induced apoptosis. Nevertheless, the mitochondrial pathway seemed to exert at least contributory functions for TRAIL-induced apoptosis in SK-Mel-13, and it revealed as essential in A-375 and in Mel-HO.

The crucial question of the relative contribution of the two agonistic TRAIL death receptors was addressed in the present study by utilizing monoclonal antibodies blocking either one of the two. Specificity of these antibodies to bind and block their appropriate targets has been shown previously (Sprick *et al*, 2002; Leverkus *et al*, 2003). Here, blocking of DR4 almost prevented TRAIL-induced apoptosis in melanoma cells positive for both death receptors, whereas blocking of DR5 was less efficient in these cells. In line with DNA fragmentation, activation of downstream signaling cascades (caspase-8, -10, -3, -7, Bid, XIAP) activated upon TRAIL treatment was strongly inhibited only after blocking DR4. These results proved, for the first time, the dominant role of DR4 in melanoma cells, once it is expressed. Thus, TRAIL-induced apoptosis in melanoma cells can be mediated by DR5, but DR4 signaling seemed to be faster and more effective.

4.1.3. Both TRAIL death receptors are expressed in primary melanomas

Standard anticancer therapies such as various chemotherapies so far have achieved only limited success for melanoma. Now, more powerful strategies may be provided by triggering proapoptotic pathways such as by TRAIL-mediated cell death (Fischer and Schulze-Osthoff 2005a). Combination of TRAIL with chemotherapy or radiotherapy has been demonstrated as highly effective in several in vivo models leading to complete eradication of human tumor xenografts from colon, breast, lung and prostate cancer (Naka *et al*, 2002; Singh *et al*, 2003; Shankar *et al*, 2005). The therapeutic effect coincided with induction of apoptosis, increased expression of Bax and Bak as well as of DR4 and DR5 (Naka *et al*, 2002; Shankar *et al*, 2005). Specific targeting of death receptors by selective agonistic monoclonal antibodies may provide an even more effective approach than TRAIL itself, as their activity may not be intercepted by decoy receptors (Yagita *et al*, 2004).

The ambiguity about the functionality of TRAIL receptors may have prevented the development of such strategies for melanoma. Despite positive findings in immune-incompetent mice, where tumor growth of melanoma xenografts have been reduced by combined application of TRAIL with chemotherapy (Chawla-Sarkar *et al*, 2003; Nyormoi *et al*, 2003), there were also several reports which do not support TRAIL-based strategies for

melanoma. For instance, only weak expression of TRAIL death receptors was found in melanoma primary cultures seen in relation to also weak responsiveness of these cultures (Nguyen *et al*, 2001; Zhang *et al*, 2004b). Especially DR4 has not been regarded as a main effector for TRAIL-mediated apoptosis in melanoma (Zhang *et al*, 1999; Hersey and Zhang 2001; Zhang *et al*, 2004b).

Strongly supporting the significance of our in vitro data, we show here, for the first time, significant expression of both DR4 as well as of DR5 in the majority of sections form nodular and superficial spreading melanoma, thus indicating that efficient DR4 signaling seen in vitro may also be characteristic for melanomas in vivo.

Thus, expression of TRAIL receptors and high efficiency of DR4-mediated apoptosis in melanoma may indicate the need to reassess the suitability of TRAIL and especially of DR4-based strategies in future melanoma therapies.

4.2.   Resistance to TRAIL-induced apoptosis caused by downregulation of initiator caspases and DR4

Unlike other death ligands, TRAIL bears the potential for targeting cancer cells in a selective way. Clinical trials with TRAIL or agonistic monoclonal antibodies for its receptors have been initiated for some solid tumors, however, so far excluding melanoma (Fischer and Schulze-Osthoff 2005b). Here we have shown that melanoma cells can be subdivided into two groups dependent on their DR4 expression: DR4-positive cells were highly responsive to TRAIL-induced apoptosis, whereas most DR4-negative (DR5-positive) cells were resistant. Furthermore, DR4 was expressed in the majority of primary melanomas in vivo, thus suggesting TRAIL or DR4-based strategies for therapeutic approaches also in melanoma.

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 and their overexpression caused TRAIL resistance in sensitive cancer cells (Zhang and Fang 2004; Zhang *et al*, 2004a; Bai *et al*, 2005). For melanoma cells, however, despite of negative correlation between basic expression of antiapoptotic proteins and resistance to TRAIL-induced apoptosis (Zhang *et al*, 1999; Chawla-Sarkar *et al*, 2002; Zhang *et al*, 2003), exogenous overexpression of Bcl-2, XIAP, c-FLIP has been shown to contribute to TRAIL-resistance (Thomas *et al*, 2000; Zhang *et al*, 2001; Zeise *et al*, 2004). Also, in the present study, we found that high resistance to TRAIL-induced apoptosis may occur when

antiapoptotic proteins such as Bcl-2, and Bcl-x_L were overexpressed in melanoma cells. No correlation was found between basic expression of XIAP and resistance to TRAIL. Thus, it is still poorly understood how TRAIL resistance occurs in melanoma cells, which are not transfected with antiapoptotic factors.

NF-κB has been suggested as a critical factor leading to TRAIL resistance in human hepatoma and pancreatic cancer cells (Trauzold *et al*, 2001; Kim *et al*, 2002). TRAIL-mediated apoptosis was enhanced when neuroblastoma cells were infected with a dominant-negative mutant of IκB kinase (IKK), which is essential for NF-κB activation (Karacay *et al*, 2004). NF-κB-dependent expression of Bcl-x_L in response to co-stimulatory signals (CD40–CD40L or CD28–B7 interactions) serves to protect B- and T-lymphoma cells from CD95- and TRAIL-induced apoptosis (Ravi *et al*, 2001).

In many cancer cell systems, antiapoptotic effects of NF-κB activation by TRAIL has been mainly proven by enhancement of TRAIL sensitivity in combination with specific or unspecific inhibitors of NF-κB. However, the only few papers showed a mechanism of how NF-κB exerted its antiapoptotic effects. Some discrepancies appeared when investigators tried to show the effects of NF-κB in TRAIL signalling. On one hand, NF-κB activation protected cells from ligand-induced apoptosis by induction of Bcl-x_L in leukaemia cells (Ravi *et al*, 2001), on the other hand, sensitivity to TRAIL-induced apoptosis was increased by NF-κB targeted expression of TRAIL death receptors in epithelial cell lines (Shetty *et al*, 2002). These discrepancies may reflect differences in the functions and in the relative amounts of the NF-κB subunits. For instance, in colon cancer cells, overexpression of the p65 subunit inhibits caspase-8, DR4, and DR5 expression, and enhances c-IAP1 and c-IAP2 expression after TRAIL treatment, whereas overexpression of cRel after TRAIL treatment enhanced DR4, DR5, and Bcl-x_S expression and inhibits c-IAP1 and c-IAP2 expression (Chen *et al*, 2003). The relative amounts of cRel and p65 seem therefore to determine whether NF-κB favors apoptosis or survival.

Along with other TNF ligands, TRAIL has been shown to trigger NF-κB activation (Ivanov *et al*, 2003). TRAIL-mediated NF-κB activation has been discussed as the cause of TRAIL resistance in Jurkat and cholangiocarcinoma cells (Ehrhardt *et al*, 2003; Ishimura *et al*, 2006). A similar relation has been suggested for melanoma, however, also TRAIL-sensitive cell lines with high basic NF-κB activity have been described (Franco *et al*, 2001), and they were also observed in the present study (Mel-HO).

4.1.2. High TRAIL-induced NF-κB activation in DR4 expressing melanoma cells

TRAIL-induced NF-κB activation may be triggered by both agonistic TRAIL receptors, as has been shown for HeLa cells (Harper *et al*, 2001). In myeloid leukemia cells and in normal keratinocytes, only DR4 contributed to NF-κB activation (Secchiero *et al*, 2003; Leverkus *et al*, 2003), whereas in Jurkat cells NF-κB is activated by DR5 (Zauli *et al*, 2005). For melanoma cells, we found here TRAIL-induced activation of NF-κB exclusively by DR4 (2/2 cell lines), unequivocally proven by selective blocking one or the other receptor. On the other hand, two melanoma cell lines and normal melanocyte cultures, which expressed DR5 but lacked DR4, did not show any NF-κB activation by TRAIL, irrespectively of their apoptosis sensitivity.

TRAIL-induced apoptosis and TRAIL-induced NF-κB correspond to two different signalling pathways with also different end points. In melanoma cells, a study based on time and dose kinetics suggested that NF-κB was activated earlier than the caspase pathway and also at lower concentrations of TRAIL (Franco *et al*, 2001). Due to these different kinetics, the authors have speculated that TRAIL may induce apoptosis only when present at high concentrations whereas low concentrations may increase apoptosis resistance. In contrast, we found that TRAIL signalling led to apoptosis at low concentrations, whereas NF-κB activation required higher concentrations of ligand. Furthermore, apoptosis and NF-κB activation after TRAIL stimulation occurred largely in parallel. Thus, in melanoma cells, DR4 triggered both high apoptosis and NF-κB activation in parallel.

4.2.2. High NF-κB is not the reason of upregulation for anti-apoptotic proteins

It is generally accepted for TNF-α, that its proapoptotic function is modulated by simultaneous activation of NF-κB resulting in an upregulation of antiapoptotic factors (Karin and Lin, 2002). Activation of NF-κB can also inhibit TRAIL-induced apoptosis, probably through transcriptional induction antiapoptotic genes. In leukaemia cells, induction of Bcl-x_L by TRAIL via NF-κB has been correlated to TRAIL resistance (Ravi et al., 2001). Similar, antiapoptotic effects of TRAIL-induced NF-κB activation have been proposed also for melanoma, however, the mechanisms remained elusive (Franco et al., 2001).

However, the hypothesis is complicated by the fact that stimulation of DR4 and of DR5 simultaneously induces apoptosis as well as activates NF-κB. It is believed that TRAIL-induced NF-κB activation is a slow and delayed process, and requires higher concentrations

of the ligand compared to that induced by TNF-α, suggesting that NF-κB induction by TRAIL may be a secondary, indirect effect (Kelley and Ashkenazi 2004). Also, up-regulation of NF-κB activity by overexpression of NIK and IKKβ or its inhibition by an IκBα mutant had no significant effect on TRAIL-induced apoptosis. Interestingly, the anti-apoptotic NF-κB response was inhibited during apoptosis by caspase-3 cleavage products, which exerted this effect in a dominant negative manner (Kim *et al*, 2005). These data suggested that activation of NF-κB by TRAIL was not sufficient to protect cells from TRAIL-induced apoptosis, and alternative mechanisms other than NF-κB activation, may account for TRAIL-resistance.

In the present study, we could not find significant changes in the expression levels of antiapoptotic proteins such as c-FLIP, Bcl-x_L, Survivin, Livin and Bcl-2 in melanoma cells upon treatment with TRAIL, thus, this is no indication of an antiapoptotic contribution of TRAIL-activated NF-κB. There were no changes in the expression levels of the same anti-apoptotic protein even after treatment of melanoma cells with TNF-α. These findings may suggest that NF-κB induced by TRAIL does not exert an antiapoptotic activity in melanoma cells after a short period of TRAIL treatment.

Several studies providing information on the functions of NF-κB made use of proteasome inhibitors, which prevent degradation of IκBs by the proteasome and in turn lead to downmodulation of NF-κB (Voorhees *et al*, 2003). Thus, proteasome inhibitors have been shown to increase TRAIL-induced apoptosis in pancreatic cancer cells (Bai *et al*, 2005) as well as in melanoma cells (Franco *et al*, 2001). On the other hand, a number of studies also indicated proapoptotic effects by proteasome inhibitors independently of NF-κB (Sayers *et al*, 2003; Fernandez *et al*, 2005). In our study, a significant increase of TRAIL sensitivity was observed in melanoma cells after proteasome inhibition however, a similar response was also seen in cells, which did not even activate NF-κB upon TRAIL treatment. As a clear indication for supplementary effects, we found that proteasome inhibition prevented TRAIL-induced downregulation of DR4.

Further understanding of TRAIL resistance may be provided by the selection of TRAIL-resistant cells (Nguyen *et al*, 2001; Ehrhardt *et al*, 2003; Jin *et al*, 2004a). After establishing this model for DR4-positive melanoma cells, we looked for changes in TRAIL-induced NF-κB activity. However, no increase was found in selected, TRAIL-resistant cells, and again, there were no changes in the expression levels of antiapoptotic proteins as compared to the parent cells.

Thus, i) TRAIL-induced NF-κB activity was found in highly apoptosis sensitive melanoma cells, ii) activated NF-κB did not trigger the expression of antiapoptotic proteins, iii) enhancement of TRAIL-induced apoptosis by proteasome inhibition was independent of NF-κB and iv) selected, TRAIL-resistant melanoma cells did not reveal enhanced NF-κB activity. According to these four lines of evidence, we may suggest that TRAIL-induced NF-κB activity does not contribute, in a critical way, to TRAIL resistance in melanoma cells.

4.2.3.   Resistance to TRAIL-induced apoptosis relates to downregulation of initiator caspases and DR4

So what then causes TRAIL resistance in melanoma cells? We tried to address this question with a cell culture model for TRAIL resistance. The initiator caspase-8 is well known as a key mediator for death ligand-induced apoptosis, and its deficiency has been reported to correlate with TRAIL resistance in other tumor models (Eggert *et al*, 2001; Fulda *et al*, 2001).

The role of the initiator caspase-10 however, was less well understood in TRAIL-induced apoptosis. In lung and breast cancer cells, caspase-10 mediated apoptosis induced by TRAIL (Kischkel *et al*, 2001), but it could not substitute for caspase-8 in Jurkat cells (Sprick *et al*, 2002).

In the present study, we found strong downregulation of both initiator caspases in DR4-positive melanoma cells selected for TRAIL resistance, and their expression came back in cells with recovered TRAIL sensitivity. As a proof of principle, transient transfection and overexpression these caspases could largely restore apoptosis sensitivity in resistant cells, which strongly supports their significant role for TRAIL sensitivity.

In clear contrast to DR4-positive melanoma cells, TRAIL selected, DR4-negative (DR5-positive) cells remained resistant after TRAIL withdrawal. By these data, a further difference between DR4-positive and DR4-negative melanoma cells became evident. Our findings on DR4-negative cells were supported recently by another set of DR4-negative (DR5-positive) melanoma cell lines, which also showed downregulation of caspase-8 and in addition downregulation of proapoptotic Bcl-2 proteins in selected, TRAIL-resistant cells (Zhang *et al*, 2006). Caspase-10 has not been investigated in these cells.

Downregulation of death receptors represents a general way for acquiring apoptosis resistance to extrinsic signals, as found for CD95 in lymphoma and in solid tumors (Li-Weber and Krammer 2003). In carcinoma cells from lung, colon and breast, resistance to TRAIL-induced

apoptosis was associated with low surface expression of DR4 (Kim *et al*, 2000; Jin *et al*, 2004a).

For melanoma, downregulation of DR5 has so far been regarded as a contributing issue in TRAIL resistance (Nguyen *et al*, 2001). In contrast, in the DR4-positive melanoma cells investigated here, downregulation of DR4 turned out to be a critical event for TRAIL resistance, indicated by loss of DR4 in resistant cells and its re-expression in cells that had recovered sensitivity. In addition, exogenous expression of DR4 in resistant cells could largely restore TRAIL sensitivity, whereas even massive overexpression of DR5 remained without any effect on sensitivity.

These data again proved the dominant role of DR4 over DR5 for TRAIL-induced apoptosis in melanoma cells. Further supporting these findings, TRAIL sensitivity could also be restored by exogenous expression of initiator caspases or DR4 in originally TRAIL-resistant melanoma cells that were not in vitro selected, indicating that loss of these factors may be a more common mechanism in melanoma cells critical for TRAIL resistance.

Resistance to TRAIL-induced apoptosis in melanoma cells seemed to be based on downregulation of initiator caspases and of DR4 but unrelated to NF-κB-driven upregulation of antiapoptotic factors. Resistance of DR4-positive cells was only transient, in contrast to DR4-negative cells, where it was not reversible. These findings may also become of some significance for clinical trials to be initiated in melanoma. Expression of DR4 and caspases seems to be clearly supportive for sensitivity. Levels of these factors may be increased by proteasome inhibition, which prevented TRAIL-induced downregulation of DR4 (shown here), by chemotherapeutics, which may upregulate TRAIL death receptors (Singh *et al*, 2003) or by interferon-γ, which was shown to upregulate initiator caspase-8 (Yang *et al*, 2003). Transient resistance of melanoma cells may also be circumvented by temporarily interrupted administrations.

In conclusion, this study proved, for the first time, the dominant role of DR4 in melanoma cells, once it is expressed. TRAIL-induced apoptosis in melanoma cells can be mediated by DR5, but DR4 signaling seemed to be faster and more effective. Strongly supporting the significance of in the vitro data, also for the first time, significant expression of both DR4 as well as of DR5 has been found in the majority of melanoma primary tumors, thus indicating that efficient DR4 signaling seen in vitro may also be characteristic for melanomas in vivo.

Finally, melanoma cells subdivide into two classes with respect to DR4 expression. For DR4-positive cells, high apoptosis sensitivity as well as NF-κB activation and development of only transient resistance became evident, thus possibly characterizing patients, who may benefit from TRAIL-based therapies. Routine investigations of the status of death receptors and initiator caspases may thus be helpful for appropriate stratification of melanoma patients.